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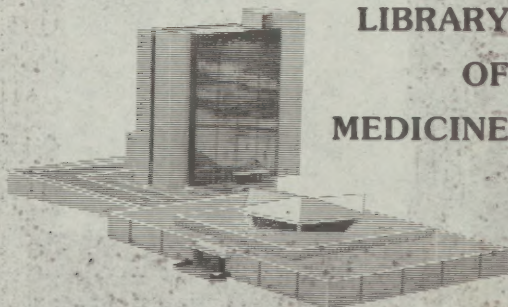
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A PRACTICAL TEXT-BOOK OF
INFECTION, IMMUNITY
AND BIOLOGIC THERAPY

WITH SPECIAL REFERENCE TO IMMUNOLOGIC TECHNIC

By

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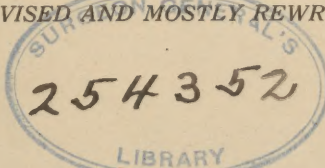
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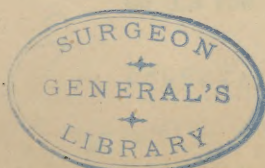
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DEDICATED TO MY WIFE

B. C. H.

WHO HAS ENABLED ME TO FIND THE
TIME IN WHICH TO PREPARE THIS EDITION

INTRODUCTION TO THE FIRST EDITION

THE last quarter of a century has witnessed an almost marvelous development of knowledge in the domain of medicine and the allied sciences, only a part, of course, of the extensive progress made in the field of general science. A striking portion of this advance has tended to broaden our knowledge of the principles and of the essential details of the processes of infection and immunity, until these branches have today come to form almost a special science in themselves—an *imperium in imperio*. Aside from the personal factor, the writer's immediate interest in the present volume, as originally projected, arose from the fact that he was desirous of having appear a series of exercises illustrative of the principles of immunology—a class-book intended to set forth in permanent form the very excellent course of instruction that Dr. Kolmer has been giving during the past few years to selected groups of interested students and occasional post-graduate workers in the Medical School of the University of Pennsylvania. That it should have surpassed the original simple plan and grown into a volume of the present proportions is scarcely to be wondered at, if the temptation to elaborate the individual exercises by explanations and cognate considerations was in the slightest to be yielded to. This is due to the fact that in its growth the subject has acquired so much of undoubted importance in the form of isolated observed facts, and itself presents so many analogies and has led to so extensive a terminology, that the author who would attempt to link the observed facts into anything like logical sequence or to add in the least to the bare cook-book-like series of illustrative exercises any explanatory paragraphs, cannot avoid the fulness that Dr. Kolmer has found inevitable in presenting the subject.

The branch of immunology, including primarily infection, and its ramifications into diagnosis and the actual treatment of disease, has brought to the parent subject of preventive medicine the greatest offering of the decades of its growth. Itself contributing to world expansion, it has nowhere found a greater stimulus than in the field of exotic pathology; and this last, in turn, has enriched internal medicine, even in its most common aspects. The first step in immunology may properly be ascribed to Jenner, with his bovine vaccine for smallpox, a step followed only after a long lapse of years by Pasteur. On the heels of the latter there appeared at once, and has since then followed, an army of men whose names crowd the history of the subject, and which many of these are bound permanently to adorn. The old vague theories of infection have taken form, and to observed facts has been added productive theory. The great danger attending this luxurious development is that, temporarily at least, the simpler, and perhaps the more obvious, facts are likely to be neglected; and, also, that symbolization by theories elaborated to harmonize with discovered facts will be accepted too fully as explanatory when in reality it does not explain, and that, as a result, investigation will finally be hampered instead of aided. In the almost universal drift of experimental studies to internal stereochemical factors, are we not in danger of placing too little stress upon actual and possible physical factors? Is there no danger that, by failing to lay

stress upon the obvious importance of the turbinate mechanism in the nose as a natural anatomic factor, our rhinologists may at least feel justified in sacrificing this mechanism too readily for what may be but trivial local reasons? Can we insist that every phenomenon described with facility in terms of the side-chain theory is really a manifestation of chemism, when perhaps, with added investigation along lines of physical absorption and the physical properties of colloids, an equally satisfying conception may be had, and possibly new facts be developed? Are we not blundering in rushing madly after matters of specificity as determined by antigen, when perhaps in reality we are confronted by potential and kinetic modifications due to peculiarities of diet or environmental circumstances? The verity of phagocytosis is open to proof by observation, and its variations are likewise to be demonstrated. Is the explanation of opsonins so convincing that merely the word itself is enough to satisfy the investigator?

Infection and immunity constitute a definite chapter in pathologic science. The processes lack the dignity of a separate science only in that they present variations, and the fact that these are glossed over by brilliant theories and conceptions cannot prevent the deliberate recognition of serious incompleteness. Yet this criticism can be applied to the growth of every branch of scientific knowledge. It in no wise militates against the right and the need for setting forth the subject in the light that, for the time, is afforded it. The importance of the criticism lies only in its acknowledgment, lest the subject as at present understood be accepted as fixed. With this danger obviated, and with all theories accepted for the time only as working theories, and their adoption not urged to curtail investigations based on other views, their prosecution can be heartily applauded.

This is the view that the writer believes that Dr. Kolmer has had in mind in his presentation of the subject as here set down. It is certainly true of the chapters that the present writer has had opportunity of examining. In such a sense, therefore, the work is urged on the appreciation of the student, whether a laboratory worker or a mere seeker of knowledge.

I have often been asked to what extent I believe it profitable to present the subject to the undergraduate student. I do not hesitate to answer that so far as the roster of the medical curriculum will permit, the laboratory demonstrations and exercises should form a part of the required course; and that, with all due caution to emphasize the fact that our present theory is not known to be final, and is offered merely tentatively, the verbal picture of the subject should be outlined before these beginners. To form some conception is necessary; and it is better, provided the mind be kept receptive, to follow a certain theory, even if it is unproved, than to do nothing at all or to work in confusion. Our American medical curriculum for undergraduates is so crowded with absolute essentials that the present subject is habitually neglected, save for a rapid lecture outline; this is an injustice to the student and to American medicine. I have tried to minimize this by providing, through Dr. Kolmer's aid, a reasonable laboratory course in the essentials of the branch to volunteer classes at first, at hours that did not interfere with the regular curriculum—at present during periods open to election. Nevertheless, the subject, influencing as it does every branch of medical practice, must take its place with other commendable additions to the required schedule. That this can be done only by lengthening the course of study, either in the annual session or by adding a year to our present four-year course, is obvious, and to that end we are rapidly approaching.

ALLEN J. SMITH.

PREFACE TO THE THIRD EDITION

To those whose continued appreciation and patronage have made necessary the preparation of this third edition, the author desires to extend his sincere thanks and to express the hope that it will receive the same generous recognition as its predecessors. More space has been devoted to the subjects of vaccine and serum therapy and the treatment of disease with non-specific protein substances. As explained later the subject of Chemotherapy has been omitted from this edition, being considered in a separate monograph now in course of preparation. For these reasons the title of this edition has been changed to Infection, Immunity, and Biologic Therapy.

As stated in the preface of the first edition, the main purposes of this book are threefold, namely:

1. *To give to practitioners and students of medicine a connected and concise account of our present knowledge regarding the manner in which the body may become infected, and the method, in turn, by which the organism serves to protect itself against infection, or strives to overcome the infection if it should occur, and also to present a practical application of this knowledge to the diagnosis, prevention, and treatment of disease.*

2. *To give to physicians engaged in laboratory work and special workers in this field a book to serve as a guide to the various immunologic methods.*

3. *To outline a laboratory course in experimental infection and immunity for students of medicine and those especially interested in these branches.*

During the six years elapsing since the second edition considerable advances have been made and especially in the fields of immunity and biologic therapy. I have found it advisable to largely rewrite the chapters dealing with these subjects with the introduction of a very large number of changes of varying importance.

The bibliographies accompanying each chapter have been greatly enriched in order to improve the book for reference purposes. Needless to state it has been impossible to include a complete bibliography on each subject, as this would have changed the essential character of the book and enlarged it too greatly.

The descriptions of immunologic methods and technic for the administration of sera, vaccines, etc., have been considerably amplified; I have endeavored to maintain the principle of describing methods with sufficient detail to make the descriptions worth while and especially helpful for the inexperienced. The chapters on precipitins, agglutinins, and complement fixation have been especially revised with this purpose in mind. References are made to the investigations of the author and his colleagues upon the standardization of the complement-fixation test in syphilis and a description of the new antigen and new method based upon these studies is included. Similar studies in complement fixation in various other bacterial and protozoön diseases and for the detection and differentiation of blood-stains, meats, and other protein substances have been completed and the results and methods are now being prepared for publication in a separate monograph in order not to enlarge the present volume too greatly.

New chapters have been added on Hemagglutinins and especially in relation to blood transfusion, and upon Serum Reactions in Syphilis Other Than Complement-fixation Reactions.

The chapters on anaphylaxis, allergy, and hypersensitiveness have been almost entirely rewritten and new chapters included on Allergy in Relation to Infection and Immunity, Clinical Allergy, Allergic Skin Reactions, Treatment of Human Allergies, and the Schick Test for Immunity to Diphtheria.

The chapters on vaccine and serum therapy have been largely rewritten and non-specific protein therapy included. New chapters have been prepared on the Principles of Active Immunization, Prophylactic Active Immunization or Vaccination in diseases of human beings and the lower animals, Principles of Passive Immunization and the Use of Sera in the Prophylaxis of Disease and the Principles of Non-specific Protein Therapy.

In the new chapter devoted to Vaccines, Sera, Blood and Non-specific Proteins in the Treatment of Disease, the administration and value of these are considered together under each disease instead of in separate chapters as is the usual custom. It is hoped that this plan will prove more helpful to practitioners by summarizing in one place under each disease what biologic therapy has to offer.

A new chapter has been prepared on the Biologic Therapy of Tuberculosis and also a new chapter on Blood Transfusion, with considerable attention to methods for transfusion, in view of the fact that the value of this form of biologic therapy has been greatly extended within recent years and the technic removed from the exclusive domain of surgery.

The part devoted to Experimental Infection and Immunity embracing a system of teaching these subjects by means of a system of experiments introduced by me in the first edition in 1915, has been enlarged by the introduction of new experiments to keep abreast of advancements in our knowledge. Experience has proved to me the value of including this teaching section in this book instead of forming a separate book, as it enables the student doing more or less independent work to consult the text for exact descriptions of technic, discussions, and theory. This plan of teaching has continued to prove a valuable aid to the author in the Graduate School of Medicine of the University of Pennsylvania and, I believe, to other teachers as well in graduate and undergraduate medical schools.

As previously stated, the subject of Chemotherapy has been omitted from this edition. So many advances have been made in our knowledge of this field of medical science that its inclusion would have enlarged the present volume too greatly. With the kind consent of the publishers I have considered this subject, including the treatment of syphilis, in a separate monograph now in course of preparation.

As stated in the preface of the first edition: "Since the larger portion of our knowledge of infection and immunity has been gained from studies upon the lower animals, it is not strange that these were early and directly benefited by a practical application of this knowledge to the prophylaxis, diagnosis, and treatment of many of the diseases to which these animals are subject. I have, therefore, included in this volume an account of those immunologic diagnostic reactions and applications of specific therapy that have a direct bearing upon veterinary medicine." In the present edition more attention and space have been given these subjects and it is hoped that veterinarians will find them adequately and helpfully discussed. Of course the fundamental facts of infection and immunity and the technic of various immunologic diagnostic methods are the same for the lower animals as for man.

Many new illustrations have been included, and it is hoped that they will serve to elucidate the text and to teach, rather than merely to embellish.

Finally, I beg again to express my deep appreciation of the unvarying efficiency and courtesy of the publishers.

J. A. K.

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INFECTION, IMMUNITY, AND BIOLOGIC THERAPY

PART I

CHAPTER I

GENERAL TECHNIC

IN this chapter simple methods are described for preparing capillary pipets and similar apparatus usually made in the laboratory, and a few general directions are given concerning the preparation of glassware and other material employed in the various methods described in succeeding chapters and in experimental work.

It may be well here to utter a word of caution to the inexperienced against observing undue haste in performing the manipulations of immunologic technic. Careful and painstaking work is essential in order to secure reliable and successful results, and should never be sacrificed for speed, the latter being attained only by experience.

CENTRIFUGE

1. A good centrifuge is one of the chief requisites of a laboratory equipment. While any good instrument will answer, preference should be given to the larger types, fitted for holding both 15 and 50 c.c. centrifuge tubes, propelled by electricity, and mounted on a concrete block in the laboratory (Fig. 1).

2. The machine must be well oiled.

3. The counter tubes should be of the same weight—it is our custom to weigh the tubes on a small balance, and adjust the counter tubes until both are of equal weight.

4. The centrifuge tubes should rest loosely upon a rubber disk or wad of cotton in the bottom of the metal tube or cup; otherwise centrifuge tubes are quite likely to be broken, especially if the machine is run at high speed.

5. The machine should be started and stopped slowly, and unnecessary speed and long running time should be avoided.

6. Never centrifugalize with cotton plugs in the centrifuge tubes. If the latter must be sealed, as when working aseptically, rubber stoppers should be used. However, if cotton plugs are large and fit tightly, they may be prevented from becoming displaced by passing through them two cross-pins in such manner that the ends will rest upon the edge of the tube. The plugs are thus prevented from being thrown to the bottom of the tube.

7. If the centrifuge is out of order, however slightly, it should not be used, but repaired at once, or else it may be ruined.

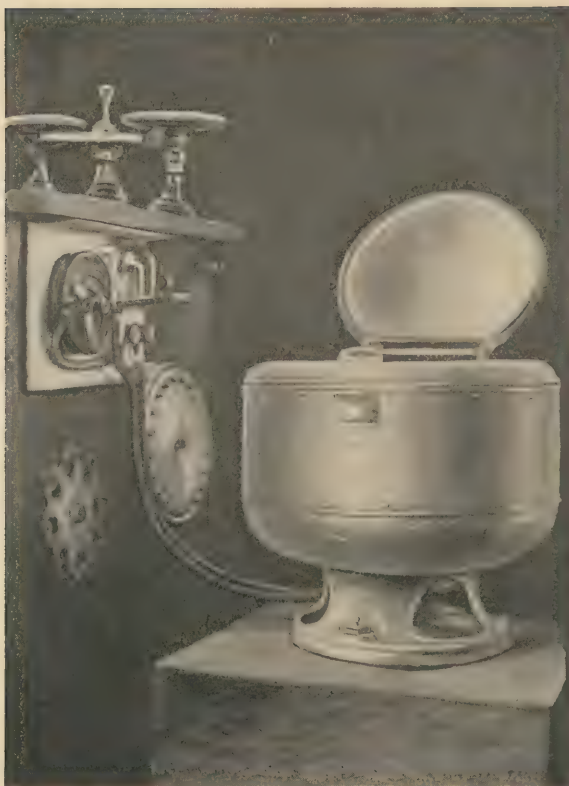


FIG. 1.—ELECTRIC CENTRIFUGE.

Mounted on a concrete block. The scales are for the purpose of weighing and counterbalancing the tubes.

PIPETS

1. Simple Capillary Pipets.—These are made of soft glass tubing in the following way:

Tubing having a caliber of 6 mm., with thin walls, that does not become opaque, brittle, or “run” on heating, and that does not contain lead, may be used. The question of alkalinity is also of importance in connection with the tubing. Many of the cheaper grades undergo disintegrative changes, which are accompanied by the setting free of alkali, especially when the glass is heated. Glass of this kind should be discarded, as it may introduce an element of error into our experiments and observations.

2. A convenient length of tubing—about 10 to 12 inches—is chosen; this will make two pipets. If a sufficient length of tubing for both sides is not available, one end may be heated and drawn out with forceps, or a handle may be added by fusing to this short end an odd piece of glass.

It is convenient to have on hand a supply of tubes cut to correct lengths, plugged at each end with a ball of cotton, and sterilized in a hot-air sterilizer. They are then ready to be drawn out as needed, thus furnishing sterile pipets with cotton plugs that tend to prevent contamination.

3. The flame must be so regulated as to play upon only so much of the tube as will suffice to furnish the glass required for drawing out the tubing.

If a Bunsen flame is used, the tip of the inner greenish flame should be applied. The margins of the flame are the hottest, and for this reason the tube must be shifted from side to side and be constantly rotated.

4. In order to secure uniform heating and satisfactory pipets the tube must be kept constantly rotated from the moment it enters until it leaves the flame. The two ends of the tube are to rest upon the middle finger of each hand, while the thumb and forefinger hold the tube in position at either side and impart the rotatory movement. It is also necessary that the tube be displaced laterally from time to time, so as to bring each portion of the middle segment of the tube in turn into the edge of the flame (Fig. 2). If the latter precaution is omitted, we shall obtain a pipet with a central bulb or thicker segment and with thinner segments on each side corresponding to the portions of the tube which lie in the edges or hottest portion of the flame.

5. The tube is heated in this manner until the glass is quite plastic. No attempt is made to draw out the tube until it has been entirely withdrawn from the flame, as otherwise a portion becomes unduly thin and plastic and divides, leaving a small, bent, and very poor pipet in each hand.

The rapidity and force with which the tube is drawn out determine the caliber of the capillary stem. By drawing rapidly a tapering capillary

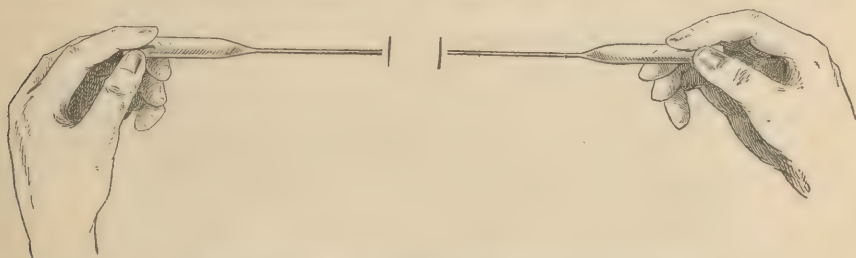


FIG. 2.—METHOD OF MAKING A SIMPLE CAPILLARY PIPET.

Shows manner of holding tubing in a flame and drawing into capillary tubes. A large portion has been removed from the center.

tube is obtained; by drawing slowly a larger capillary tube of more uniform caliber is obtained. Of course, the worker cannot take too much time, as the glass hardens quickly. With a little practice this part of the technic is soon mastered. Thorough and uniform heating and careful, steady pulling when the tube is sufficiently plastic are of primary importance.

When, owing to an error in judgment in heating the tube, it is withdrawn before it is sufficiently plastic and begins to harden, the situation cannot be remedied by drawing out the tube quickly with a jerk. Similarly, when a tube has been partially drawn and hardens it cannot, as a rule, be reheated and drawn out to make a satisfactory pipet.

6. After drawing out the pipets the hands should be held steady for a few seconds, *i. e.*, until the glass has hardened; otherwise the tubes will bend and be less satisfactory.

7. Capillary pipets are manipulated with *rubber teats*, which should be of the best soft vulcanized rubber, and should fit snugly upon the pipet, rendering it air-tight.

2. **Looped Pipets.**—Looped pipets find their main application in the measurement of the bactericidal power of the blood, after the method of Sir A. Wright.¹

¹ Technique of the Teat and Capillary Glass Tube, 1912. Constable & Company, London.

The essential features of these pipets are: (a) The capillary stem, which serves for measuring and mixing the bacterial emulsion and serum; (b) the portion that serves first as a chamber for the sterile nutrient broth and later as a cultivation chamber for determining whether the microbes that have been mixed with the serum have or have not been killed by it; (c) the glass loop, which acts as a trap, preventing extraneous contamination, and (d) the handle, upon which the rubber teat can be fitted. With a little practice these pipets are readily made.

1. Select glass tubing about 6 inches in length.

2. Heat one portion about 2 inches from the end, and when sufficiently plastic, draw it out for about 2 or 3 inches or until it is long enough to give a spiral loop of the desired dimensions (Fig. 3). While the glass is still plastic hold the left hand steady, and with the right hand lower the tubing and make a spiral loop in such manner that the loop is closely applied, but does not touch the sides of the upper and lower segments of the tube. Actual contact with the sides must be avoided, for this would produce strain and predispose the tube to fracture.

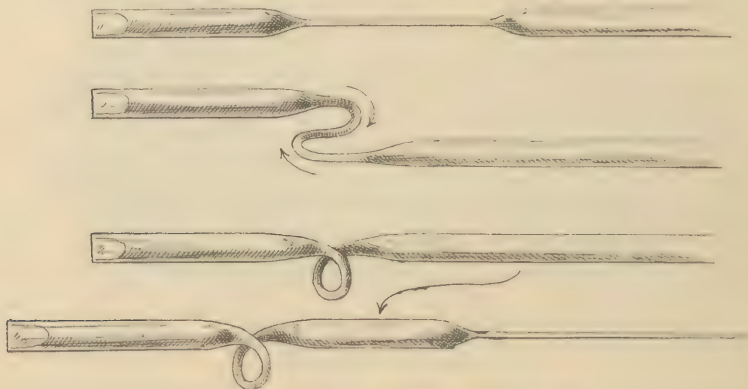


FIG. 3.—METHOD OF MAKING A LOOPED PIPET.

3. The longer portion of tubing is now heated and drawn out to a capillary pipet and broken through at the desired point.

4. Instead of this method the capillary portion may be drawn before the loop is made.

3. Graduated Pipets.—1. In this work 1 c.c. pipets graduated into $\frac{1}{100}$ c.c.; 5 and 10 c.c. pipets graduated into $\frac{1}{10}$ c.c. will render satisfactory service. The pipets should be calibrated to the tip, and should preferably be long, with a narrow lumen, rather than short with a wide lumen, as the latter renders the markings too close to one another. For pipeting small amounts, as in certain complement-fixation tests, a 0.2 c.c. pipet graduated to $\frac{1}{100}$ c.c. will be found quite serviceable, permitting accurate measurement of small amounts of fluid. The entire length of these pipets is equal to the ordinary 1 c.c. pipet which renders the subdivisions far apart and quite easy to read. These pipets are made by competent dealers upon special request.

2. These pipets should be perfectly clean and clear, sterilized, and have sharp, easily read markings. Pipets with broken tips are difficult to handle, and if calibrated to the tip are inaccurate.

PIPETS

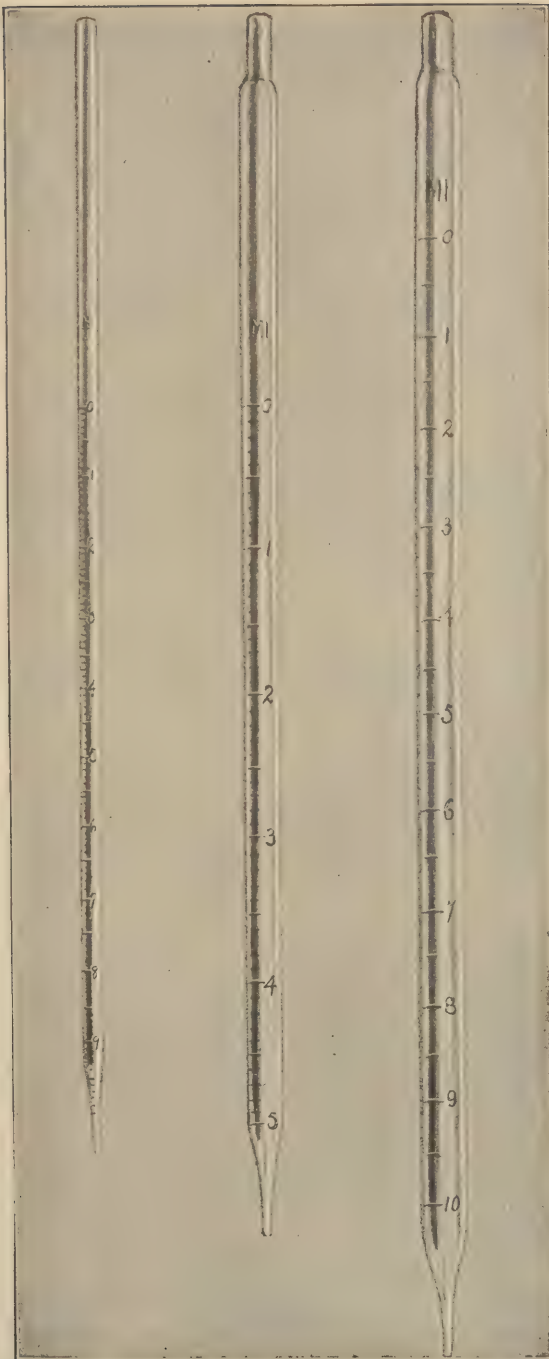


FIG. 4.—GRADUATED PIPETS. (American Jour. Syphilis, 1922, 6, 92.)

3. The worker should practice methods of making accurate measurements. The slightest slip may mean an inaccurate measurement and produce untoward results. The mouth end and the pipeting finger should be dry, otherwise on measuring small amounts the delivery will be jerky and usually unsatisfactory.

4. After pipets have held infectious material they should be placed at once in a jar containing 1 per cent. formalin solution. After pipeting blood, milk, or serum the pipets should be rinsed or placed in a jar containing water or a weak lysol solution, as the formalin solution tends to harden these substances and renders cleaning quite difficult.

5. The jar for holding soiled pipets should contain a layer of cotton, otherwise the tip may be broken off when the pipets are dropped in.

Buck¹ has recently devised a multiple pipet capable of delivery into 12 test-tubes simultaneously; this pipet is especially serviceable in conducting large numbers of agglutination and complement-fixation tests.

BLOOD CAPSULES

Blood capsules were devised by Sir A. Wright for collecting small amounts of blood for examination. The essential features of a capsule are: (a) The upper straight limb which can be drawn out to serve as a needle for punc-

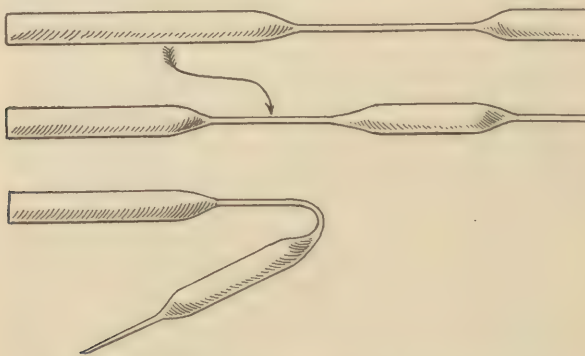


FIG. 5.—METHOD OF MAKING A WRIGHT BLOOD CAPSULE.

turing; (b) the recurved limb which makes it possible to fill the capsule by gravity without risk of the inflow being arrested by the blood running down and blocking the straight limb which provides an outlet for the air.

These capsules are easily made and prove quite serviceable, especially for collecting small amounts of blood for making agglutination tests, opsonic measurements, etc.

1. Take a piece of soft glass tubing about 10 or 12 cm. in length, and having an internal diameter of at least 5 mm.

2. Draw one end out into a capillary stem, and break this at an appropriate point (Fig. 5).

3. Then reinsert the tube into the flame, and leaving a portion at least 3 cm. in length to serve as the barrel of the capsule, draw out the tube into a capillary stem about 8 cm. in length, and bend it so as to form a stout recurved limb lying in the horizontal plane; now, before the glass has lost its plasticity, draw the capsule gently upward so that its long axis will be at an angle of about 30 degrees horizontally. Finally, separate the capsule

¹ Jour. Infect. Dis., 1916, 19, 267.

from the main tube by filing it across the capillary portion at the distance indicated in the accompanying illustration (Fig. 5).

4. The straight limb may now be drawn to a sharp point and used as a needle.

Test-tubes may be drawn out and converted into ampules for holding vaccines, serums, or other fluids.

1. *Thin-walled* and sterilized test-tubes of appropriate size are chosen.

2. The tube is heated at a point near the open end in the Bunsen flame in the same manner as the glass tubing, *i. e.*, by keeping the tube constantly rotating with a lateral movement to insure uniform heating.

3. When the glass has become plastic it is drawn out into a stout stem.

4. After cooling, it is filed through at an appropriate place, being careful to leave a somewhat long stem (Fig. 6).

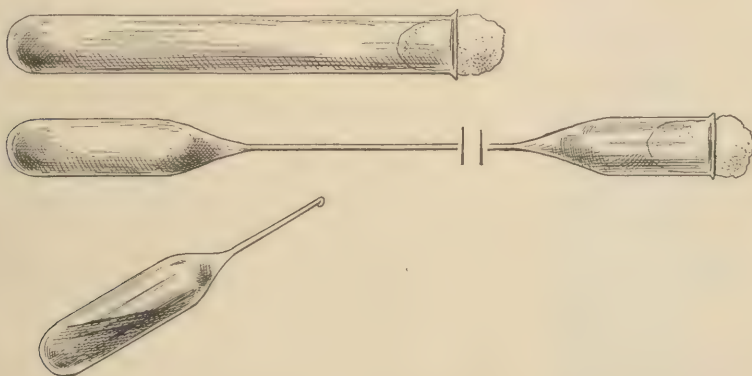


FIG. 6.—METHOD OF MAKING A LARGE VACCINE AMPULE OF A TEST-TUBE.

5. The open end may now serve as a funnel for filling the bulb.

6. The bulb is now sealed by warming the air above the level of the fluid and then sealing the tip. With a long stem, in order to secure a portion of the contents, the sealed end may be broken off from time to time; it is readily resealed.

7. Instead of this procedure the fluid may be placed in the test-tube at once, the upper end being heated in the usual manner and drawn out; the stem is broken through and the tip sealed. If the tube is small or the contents are such as will almost fill a tube, this method may not be successful, owing to the production of steam on heating the fluid, which either cracks the bulb or causes the tip to explode at the time of sealing.

TEST-TUBES

Different tests require test-tubes of varying sizes according to the nature of the work; as a general rule the width should be such as permits mixing the contents without capping the tube and inverting. The various sizes which I have found useful are described with the different methods.

Test-tubes should be made of good glass with no lips and with round bottoms; they should be well annealed.

CLEANING OF GLASSWARE

1. All glassware used in immunologic tests should be *physically and chemically clean*; this is especially true of test-tubes, pipets, and flasks used

in complement-fixation work. As shown by Hektoen and Ruediger,¹ Manwaring,² Cumming,³ Brown, and myself⁴ traces of acids and alkalies may prove destructive for complement or hemolytic, and must be carefully avoided in all glassware and solutions.

2. All glassware, including pipets, test-tubes, and flasks, should be brilliantly clear and glistening, and the markings distinct and easily read.

3. Unless infectious material has been used it is not generally necessary to boil the glassware; repeated boiling in soapy water tends to cloud the glass and render it unsightly. When this occurs it may be improved by immersion in 2 per cent. hydrochloric acid or the bichromate cleansing fluid (2 parts potassium bichromate, 3 parts commercial sulphuric acid, and 25 parts water) for twenty-four hours, followed by *thorough rinsing in running water*.

4. After use tubes are emptied; rinsed in running tap-water; washed inside and outside in a pan or bucket of warm soapy water; thoroughly rinsed in running tap-water and placed upside down in metal baskets, and heated in a hot-air oven until a piece of fresh cotton placed in the oven has turned a light brown color. It is unnecessary to plug each tube with cotton, although it is advisable to plug the flasks because of their use during subsequent work. In this connection mention may be made of the report of Langer,⁵ who found that cotton may contain antilytic substances, and to warn against permitting blood, complement serum, or other reagents to soak into cotton stoppers by reason of the possibility of thereby dissolving out anticomplementary material. In some laboratories it is customary to boil the tubes in soapy water; rinse in tap-water; immerse briefly in 1 per cent. hydrochloric acid to neutralize the alkali of the soap; rinse thoroughly, and sterilize. I believe the boiling and immersion in acid are unnecessary as routine procedures, although it is occasionally necessary to do this with tubes which are spotted and particularly dirty; *the rinsing after immersion in the acid bath must be particularly thorough in order to remove all traces of acid*.

5. New test-tubes should not be used until thoroughly washed with soapy water, rinsed, and sterilized as described above. In some laboratories it is customary to give them an acid bath as described.

6. While it is preferable to prepare the glassware on the day preceding the tests, the tubes are fit for use any time within a period of several days after preparation.

7. Pipets are cleaned in the same way and placed in metal boxes or wrapped in newspaper and sterilized in the hot-air oven. The mouth ends may be plugged neatly and firmly with a bit of cotton.

SYRINGES

A good syringe is indispensable in performing bacteriologic and immunologic work. Various sizes should be at hand, but usually a graduated 5 c.c. syringe answers most purposes.

1. Many kinds of syringes are on the market. Those with rubber or leather plungers and packings are unsatisfactory, as they cannot be sterilized by boiling, and soon leak. Nothing is more exasperating than a leaking syringe, as with the leakage unknown quantities of inoculum are lost, not

¹ Jour. Infect. Dis., 1904, 1, 379.

² Ibid., 1904, 1, 112.

³ Ibid., 1916, 18, 151.

⁴ Amer. Jour. Syph., 1919, 38.

⁵ Deutsch. med. Wchnschr., 1913, xl, 274.

to mention the possible dangers of contaminating the fingers, the animal, and the laboratory.

Syringes with metal or glass plungers are to be preferred, as are also those upon which the needle may be fitted without screwing (Fig. 7).

2. The old Koch syringe is fitted with a rubber bulb for filling and expelling the fluid. This arrangement is well adapted for making subcutaneous injections, but is somewhat dangerous for purposes of making intravenous injections on account of the danger of injecting air.

3. Syringes may be sterilized by filling them with 1 per cent. formalin solution for a few minutes, followed by several washings with sterile water or salt solution. This method is good for syringes having leather or rubber packings and plungers. It is not safe for blood-cultures, as spore-forming bacteria may escape the sterilizing process.

4. With all glass or metal syringes it is best to boil the syringe, especially if a careful aseptic technic is to be employed. The plunger should be removed from the barrel, or else, whether it be of glass or metal, it will expand more rapidly than the accommodation of the barrel will permit. All parts should be placed in a pan or wrapped in gauze, warm water added, and boiling allowed to take place for a minute or so. After cooling the parts are adjusted.

5. If infectious material has been used the syringe, after using, should be washed out and sterilized. The needles should be dried and wired, and a small amount of vaselin rubbed over to prevent rusting. The plunger may likewise be occasionally rubbed with a small quantity of vaselin. Needles may be kept in oil or in absolute alcohol; usually thorough drying and wiring preserves them in good condition.

SOLUTIONS

As a general rule, freshly distilled water should be employed, and particularly in those localities where limestone and other alkalies abound.¹

Physiologic Saline Solution.—Sodium chlorid (0.85 per cent.) in distilled water is best adapted for immunologic work. This solution may be prepared as follows:

1. Keep C. P. sodium chlorid in a tightly stoppered bottle; if sufficient moisture has collected to render the salt somewhat lumpy, dry a portion in the hot-air oven for ten or fifteen minutes before weighing.

2. Weigh out 8.5 grams and dissolve in 1000 c.c. of freshly distilled water in a chemically clean and dry flask furnished with a gauze-covered cotton stopper; filter through a good paper.

3. Sterilize by heating in an Arnold for an hour; smaller bulks require

¹ Amer. Jour. Syph., 1919, 3, No. 1.



FIG. 7.—A SATISFACTORY SYRINGE (Record).

This syringe has a glass barrel and metal plunger. It is easily sterilized, durable, and works smoothly and accurately.

less heating. (Do not sterilize in an autoclave in order to avoid possible concentration of salt by loss of water in steam.)

4. Before using in the Wassermann reaction it is well to test the tonicity by adding a drop of washed blood-cells to 5 c.c. of the solution in a test-tube; if there are no immediate signs of lysis or none after gentle mixing and standing aside for half to an hour, the solutions may be accepted.

Sodium Citrate Solution.—This is prepared in 1 : 10 per cent. solution, using physiologic saline solution and not plain or distilled water. This solution is employed for the preventing of coagulation of blood and inflammatory exudates.

CHAPTER II

METHODS OF OBTAINING HUMAN AND ANIMAL BLOOD

As a general rule, when blood is withdrawn to obtain serum a careful aseptic technic should be employed. Similarly, when erythrocytes are to be obtained for purposes of immunization it is necessary to avoid contamination by proper cleansing of the parts, and the use of sterile needles, containers, and solutions. In obtaining erythrocytes for making hemolytic tests it is not necessary that the blood be absolutely sterile, the ordinary precautions against gross contamination being sufficient.

Blood may be withdrawn to obtain the corpuscles or serum, or both.

OBTAINING ERYTHROCYTES

Red blood-corpuscles are usually obtained and washed free of serum for the purpose of making complement-fixation and other hemolytic tests. For these purposes three methods are commonly employed, namely: (a) Bleeding into a suitable vessel, and defibrinating with glass beads or rods; (b) bleeding into an anticoagulating fluid, and (c) breaking up coagula of blood and thereby liberating erythrocytes. The latter method is frequently used for securing human cells from specimens of blood submitted for the Wassermann reaction.

In a comparative study of these methods Brown and myself¹ have found that the method of collection has but slight influence upon the erythrocytes, provided the corpuscles are washed one or more times to remove all traces of liberated hemoglobin and serum. The following methods are satisfactory:

1. Blood is collected in a sterilized flask or Mason jar containing glass beads or broken glass rod, and gently shaken for five or ten minutes; the blood is now filtered through a bit of cotton to remove particles of fibrin.

2. Blood is collected in a flask containing 1 per cent. solution of sodium citrate in physiologic saline solution, or water, allowing at least 1 part of citrate solution to 4 parts of blood. Dog corpuscles are quite fragile, and Rous and Turner² have found that the addition of $\frac{1}{8}$ per cent. gelatin to the citrate-saline solution aids in protecting the cells. In collecting small amounts of blood it is better and more economical to use one of these anticoagulating fluids.

WASHING ERYTHROCYTES

For purposes of immunization or in making hemolytic tests red blood-corpuscles are washed free of serum before being used for the following reasons:

- (1) Avoid possible precipitin reactions; (2) to avoid possible anti-complementary activity of the animal's serum if the blood is more than a day old; (3) to avoid anaphylactic reactions if the cells are used for purpose of injecting rabbits at long intervals in the preparation of hemolysin, and (4) to avoid erroneous Wassermann reactions if human cells are being employed by carrying over serum with corpuscles.

¹ Amer. Jour. Syph., 1919, 3, 169.

² Jour. Exper. Med., 1916, 23, 219.

1. Place the citrated blood, which has previously been diluted with sufficient salt solution, in centrifuge tubes. Defibrinated blood may be placed in centrifuge tubes, and 5 to 10 volumes of sterile normal salt solution added and thoroughly mixed. Tubes are then carefully balanced and centrifuged at moderate speed for five minutes.

2. Remove the supernatant fluid down to the corpuscles with a sterile pipet. Add an equal volume of salt solution; mix the corpuscles and centri-

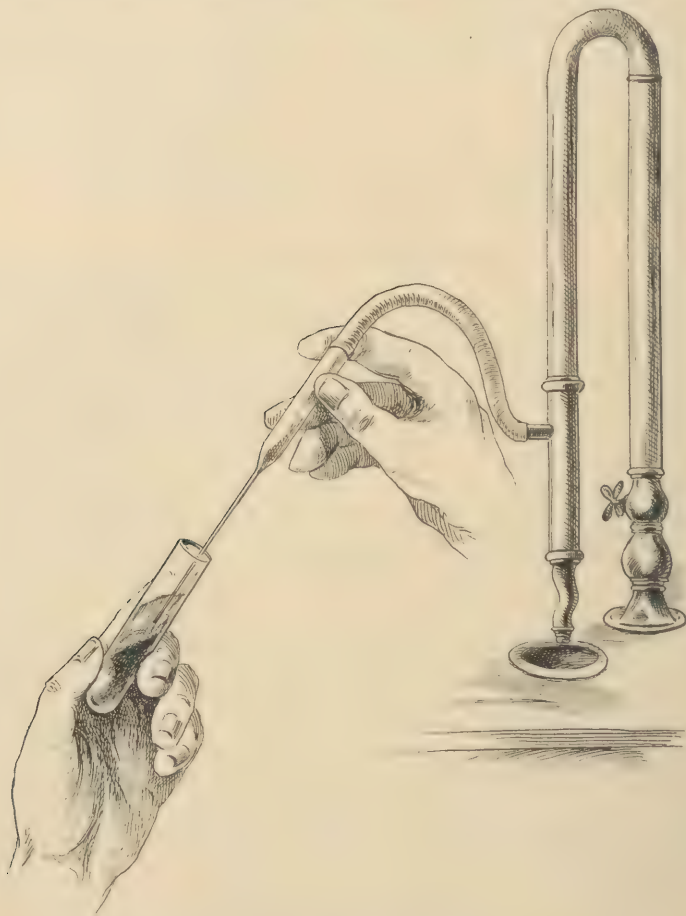


FIG. 8.—A SUCTION PUMP.

By attaching a capillary pipet to the rubber tubing fluid may be removed without disturbing the sediment.

fuge. This process should be repeated once more in order to insure thorough washing of the corpuscles to remove all traces of serum. For removing supernatant fluids which are to be discarded the suction pump shown in Fig. 8 will be found very useful. It is well to fit the rubber tubing with a capillary pipet which permits the supernatant fluid flush with the sediment to be removed.

3. After the last washing the supernatant salt solution should be carefully removed when the corpuscles are ready for use.

PRESERVATION OF ERYTHROCYTES

For the sake of necessity, economy, or convenience it may be necessary in certain laboratories to attempt the preservation of the blood used in the preparation of hemolysins and suspensions for complement-fixation tests. Two methods have been tried, although the great majority of workers prefer the use of fresh blood: the formalin method of Bernstein and Kaliski,¹ also suggested by Armand-Delille and Launoy,² consisting in adding 0.5 c.c. of formalin (40 per cent.) to 400 c.c. of defibrinated blood (approximately 1 : 800 dilution of formalin), and the method of Rous and Turner,³ consisting in collecting blood in Locke's solution containing 1 per cent. sodium citrate in the proportion of 1 part of blood to 4 parts of solution, washing three times in Locke's solution containing 0.25 per cent. gelatin, and preserving in Locke's solution +2.8 per cent. saccharose.

Bernstein and Kaliski found that defibrinated sheep blood preserved with 1 : 800 formalin proved satisfactory for two months, whereas plain defibrinated blood was generally unfit for use after seven days; also that blood collected in a 1 per cent. solution of ammonium oxalate as an anticoagulant, followed by the addition of formalin to 1 : 800, kept as well as defibrinated blood with the same amount of formalin. These investigators found that formalin in dilution as low as 1 : 200 was without effect upon the Wassermann reaction, and that formalized cells could be used for the immunization of rabbits in the production of antish sheep hemolysin. Experiments with citrated human blood with the addition of formalin to 1 : 400 dilution yielded satisfactory results over a period of four weeks. Reinmann,⁴ in a study of both methods with sheep blood, found that formalized cells (Bernstein-Kaliski) were satisfactory for four weeks, and that saccharose-preserved cells (Rous-Turner) were satisfactory from twenty-one to twenty-five days when kept in sealed ampules.

After a comparative study of these methods Brown and myself⁵ found the following methods satisfactory:

1. Plain, defibrinated blood kept at a low temperature; a suitable jar supplied with glass beads or small pieces of glass rod and a cover is sterilized with dry heat, and used for the collection of blood at an abattoir or by venupuncture of a sheep. After defibrinating by shaking the whole is kept at 2° to 4° C., leaving the defibrinated blood with the fibrin clot.

2. Formalized blood after the method of Bernstein and Kaliski kept at a low temperature. Defibrinated blood collected as described is filtered through a small wad of absorbent cotton into a sterile flask, and 0.1 c.c. of pure formalin (39 to 40 per cent.) added to each 80 c.c. of blood; or the blood may be collected in 1 per cent. sodium citrate in physiologic saline, or Locke's solution, in the proportion of 1 part blood to 4 of solution, and formalized in the same manner. For small amounts of blood a 1 : 10 dilution of formalin in physiologic saline solution (1 c.c. of formalin and 9 c.c. saline solution) may be used in amount of 0.1 c.c. for each 8 c.c. of blood. This is the simplest and most practical method for the preservation of cells over a period of two to four weeks.

Preserved blood tends to become dark in color and the cells increasingly fragile; it should not be used unless the following two conditions at least are fulfilled:

¹ Ztsch. f. Immunitätsf., 1912, 13, 490.

² Ann. d. l'Inst. Pasteur, 1911, 25, 222.

³ Jour. Exper. Med., 1916, 23, 219.

⁴ Jour. Lab. and Clin. Med., 1916, 2, 200.

⁵ Amer. Jour. Syph., 1919, 3, 169.

1. When washed with 3 or more volumes of physiologic saline solution by centrifugalization there should be no discoloration of the supernatant fluid after the second washing.
2. The blood should become of a brighter and normal red color.

OBTAINING BLOOD-SERUM

If serum is desired at once, blood should be drawn into sterile centrifuge tubes, and the tube immersed in cold water for from five to ten minutes; this facilitates clotting. The clot is then broken up with a sterile platinum wire or glass rod, and the serum secured by rapid centrifugalization. Or blood may be drawn into sterile cylinders, Petri dishes, or centrifuge tubes, and allowed to stand at room temperature for a few hours, after which they should be placed in a refrigerator until the serum separates. Blood never should be drawn into Erlenmeyer flasks because of the difficulty of drawing off serum without disturbing the clot. When drawn into Petri dishes, care should be taken that the layer of blood is not too thin, otherwise drying will occur with poor separation of the serum. As a rule, the best results are secured by placing blood in centrifuge tubes, for if separation is poor or does not occur at all, the clot may be broken up and serum secured by centrifugalization. So far as possible, avoid drawing blood from an animal immediately after feeding, as under these circumstances the serum is likely to be milky or opalescent.

OBTAINING CORPUSCLES AND SERUM

For certain purposes it may be desirable to obtain both serum and corpuscles; these may be secured in the following way:

1. Place blood in a large centrifuge tube or cylinder, and defibrinate with rods or glass beads.
2. Centrifuge thoroughly.
3. Remove the serum, which is slightly discolored on account of defibrination, with capillary tube and rubber teat.
4. Filter the corpuscles into a centrifuge tube through a wisp of cotton in a funnel to remove small particles of fibrin.
5. Add normal salt solution, and proceed with the washing process.

OBTAINING BLOOD PLASMA

In obtaining blood plasma it is necessary to avoid coagulation of blood by securing and handling the blood with the least amount of trauma to leukocytes (paraffined tubes), and centrifuging rapidly and at once.

In the paraffin method devised by Freund, centrifuge tubes are coated with paraffin and chilled to a low temperature in order to prevent coagulation and the collection of plasma. In my experience this method has proved unsatisfactory, inasmuch as the plasma regularly coagulated when brought to body temperature.

Meeker has devised a method consisting in marking an appropriate centrifuge tube at 30 c.c. and placing within 0.75 c.c. of a 2 per cent. solution of sodium oxalate, followed by drying in a gas flame with even distribution of the oxalate up to the fiduciary mark, but avoiding boiling. He found that 0.0005 gram sodium oxalate was sufficient to decalcify 1 c.c. of human blood. Blood was then collected from a congested vein up to the 30 c.c. mark followed by admixing with the powdered oxalate adhering to the walls of the centrifuge tube, and immediate centrifugalization.

With this method it was not always possible to avoid having blood

come in contact with the unprotected glass above the mark, or to secure even mixture of blood and oxalate; for these reasons partial coagulation not infrequently occurred with the production of serum.

These faults were corrected in the following method devised by Watanabe¹ in my laboratory, which is a combination of the paraffin tube and Meeker methods:

1. A centrifuge tube is marked to indicate a volume of 20 c.c., and 1 c.c. of a 2 per cent. solution of sodium oxalate added; this amount of oxalate is double that used by Meeker and effectually prevents coagulation.

2. The solution of oxalate is then dried as evenly as possible in the test-tube held over a gas flame up to, and a little beyond, the 20 c.c. mark.

3. The upper portion of the tube is now coated with a thin layer of molden paraffin by means of a small soft brush so that there is no unprotected glass.

4. Blood is collected by means of a short, wide bored needle into the prepared tube which is gently rotated during and immediately after the collection of 20 c.c. and immediately centrifuged for thirty minutes at high speed. The supernatant plasma is then pipeted to a second plain centrifuge tube and centrifuged at high speed for two to two and a half hours. The resulting plasma is free of leukocytes, and almost or entirely free of blood-platelets.

OBTAINING SMALL AMOUNTS OF HUMAN BLOOD

For obtaining small amounts of blood—up to 2 or 3 c.c.—for the Widal reaction, complement-fixation, and other tests the following method is satisfactory:

1. Wash the last joint of the middle finger with alcohol. If the hand is cold, it should be warmed by immersing it in hot water. Before puncturing compress the finger and squeeze in such a manner as to drive the blood toward the end of the finger.

2. Prick deeply with a broad blood lancet, Hagedorn needle, or scalpel (Fig. 9).

3. Collect the blood in a small test-tube—about 8 by 1 cm.—such as is used in performing the Noguchi reaction for the serum diagnosis of syphilis (Fig. 10).

4. By squeezing the finger sufficient blood can usually be obtained from one puncture practically to fill a tube of the size mentioned. One to 2 c.c. of serum are easily obtained in this manner, and this is sufficient for conducting the ordinary serum reactions. When the treatment of syphilis is being guided by the Wassermann reaction, frequent tests are necessary, and a patient may object to submitting to repeated venipuncture. The method for securing blood just described is so simple and efficient that objections to it are never made.

5. Blood may also be drawn in a Wright capsule, made by drawing out ordinary thin glass tubing in the Bunsen burner (see p. 4). After sufficient blood has been collected (Fig. 11), the straight empty end is sealed with a flame and then cooled (Fig. 13). The blood is then shaken into this sealed end and the bent end, in turn, sealed with the flame. Care should be taken not to heat the blood. When the serum has separated the tube is opened by filing at a point above the clot and breaking, protecting the hands with a towel. The serum is carefully removed with a capillary pipet and nipple (Fig. 12).

¹ Jour. Immunology, 1919, 477.



FIG. 9.—METHOD OF PRICKING A FINGER.

The patient's finger is grasped firmly and lanced with a Daland lancet *across the folds of skin*. When lanced parallel with the skin-folds the wound is likely to close before sufficient blood is secured.

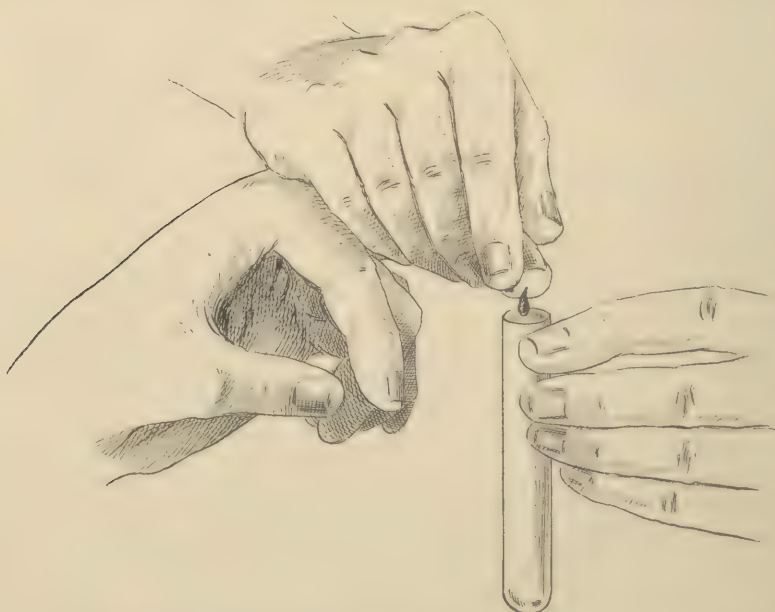


FIG. 10.—METHOD OF SECURING A SMALL AMOUNT OF HUMAN BLOOD.

By pricking the finger deeply *across* the lines of the skin with a *broad* lancet two or more cubic centimeters of blood are easily collected in a small test-tube. Do not use a large tube, as blood may be lost on the sides of the tube.



FIG. 11.—COLLECTING BLOOD IN A WRIGHT CAPSULE.

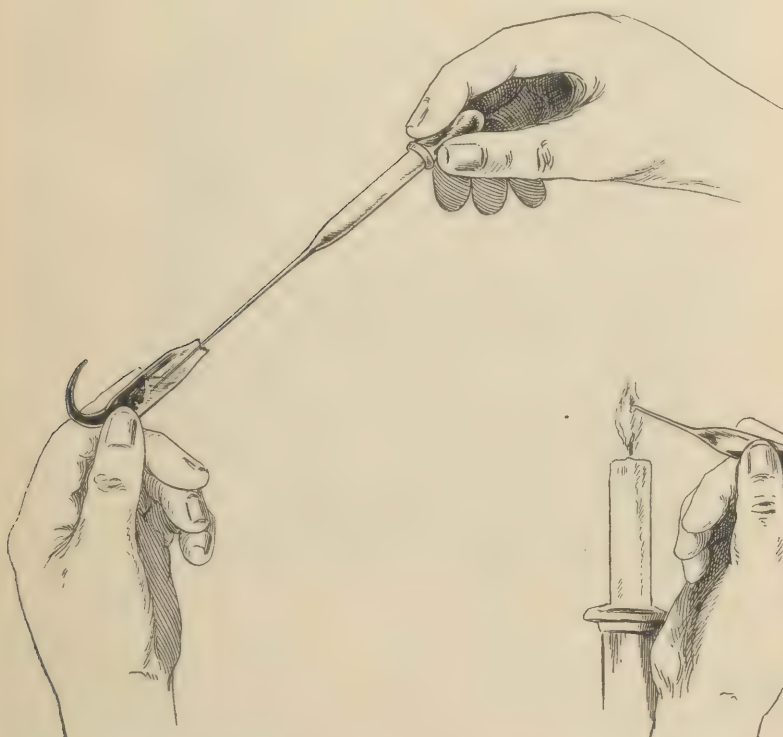


FIG. 12.—REMOVING SERUM FROM A WRIGHT CAPSULE.

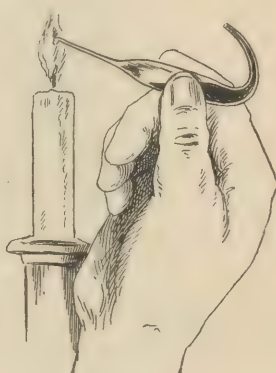


FIG. 13.—METHOD OF SEALING A WRIGHT CAPSULE.

6. To obtain blood from infants and small children the large toe may be punctured, but, as a rule, better results are obtained by wet cupping or by puncturing a vein.

OBTAINING LARGE AMOUNTS OF HUMAN BLOOD

Larger quantities of human blood may be required for making complement-fixation reactions, the Abderhalden ferment test, etc.

(a) **Phlebotomy.**—1. In adults a prominent vein at the elbow, such as the median basilic, is usually chosen. In children less than a year old



FIG. 14.—METHOD OF OBTAINING BLOOD BY VENIPUNCTURE FOR SEROLOGIC TESTS.
(Keen's Surgery.)

A tourniquet of garter elastic is being employed with a slip knot; above, a plain No. 16 needle grasped with a hemostat has been entered into a vein; below, a Keidel tube is being used, with a hemostat in position to crush the stem of the ampule.

this vein is not suitable, better results being obtained when the external jugular or a temporal vein is used (Fig. 18).

2. Place a rubber tourniquet or a few firm turns of a wide muslin bandage above the elbow. I can recommend the use of ordinary garter elastic adjusted with a slip knot as shown in Fig. 14.

3. Apply tincture of iodine to the skin over the vein. The vein may be

rendered more prominent by directing the patient to open and close the hand several times.

4. Steady the skin over the vein, and insert the needle in the direction of the blood-current (Fig. 15). It is more awkward and of no practical



FIG. 15.—METHODS FOR SECURING BLOOD BY PUNCTURE OF A VEIN.

The middle figure shows distention of the veins of the arm about the elbow. The needle is entered by a quick upward thrust. Practically any prominent and firm vein may be used. The upper left-hand figure shows collection of blood in a test-tube. Usually 10 c.c. or more are easily collected before clotting occurs. To secure large amounts use a larger needle with a *smooth bore* (preferably a platinum-iridium needle). The lower right-hand figure shows collection of blood in a Keidel tube.

advantage to puncture in a downward direction toward the hand. The needle should be sharp and of a size midway between the ordinary hypodermic and a large antitoxin needle, as the former is too small and the latter is unnecessarily large; gage No. 18, shown in Fig. 15, is quite satisfactory.

The blood is then allowed to drop into a sterile tube. It is not necessary to attach a syringe, although 5 to 10 c.c. of blood are obtained more quickly by this means on account of the possible gentle suction. Needle and syringe should be sterilized by boiling. When larger quantities of human serum are required, as in autoserum therapy, a platinum-iridium needle should be used, as coagulation in the needle is less likely to occur; besides, these needles are readily sterilized by heating in the flame.



FIG. 16.—THE KEIDEL TUBE FOR COLLECTING BLOOD.

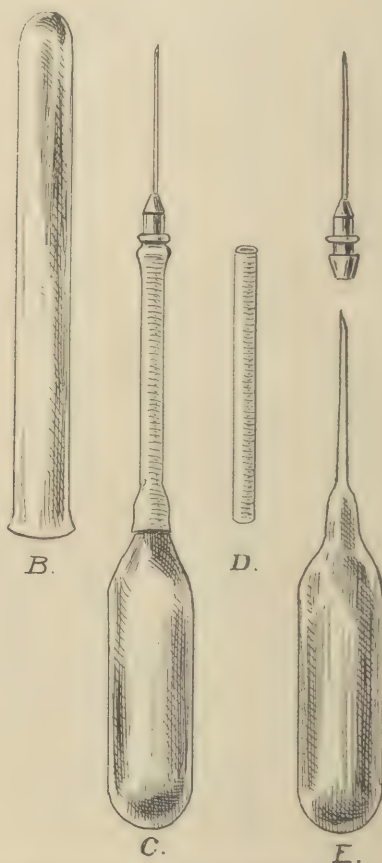


FIG. 17.—PARTS OF THE KEIDEL TUBE.

E is the vacuum bulb which is attached to the needle by a piece of rubber tubing; (*D*); the glass tube (*B*) covers the needle and the whole is sterilized.

5. Loosen the tourniquet, withdraw the needle quickly, and seal the wound with a touch of flexible collodion.

6. Instead of a syringe the 5 c.c. vacuum bulb devised by Keidel has proved quite satisfactory (Fig. 16). This apparatus consists of a 5 c.c. ampule with arm drawn out to a capillary tip and sealed after a vacuum has been created by heating (Fig. 17, *B*). A short piece of rubber tubing



FIG. 18.—METHOD OF OBTAINING BLOOD FROM AN ANTERIOR JUGULAR VEIN. (Keen's Surgery.)

The patient is a child six years of age; shows the position of the patient and manner of distending the vein by pressure above the clavicle. A 5 c.c. Record syringe fitted with a No. 20 needle is being used for the withdrawal of blood. The distended vein is painted with tincture of iodine to indicate the position.



FIG. 19.—A WET CUP FOR SECURING BLOOD FROM CHILDREN. (Devised by Blackfan.)

The cup is held in this position over a scarified area; air is exhausted by means of a pump attached to the rubber tubing; blood collects in the small test-tube. (Made by Hynson, Westcott & Dunning, Baltimore, Md.)

connects the needle and the capillary portion of the ampule. A needle of No. 25 gage is fitted tightly into the free end of the rubber tubing. A slender

glass tube closed at one end and flaring slightly at the other serves as a protection for the needle, which it covers when the apparatus is sterilized. The apparatus is sterilized in a hot-air oven at 150° C. for one hour. To obtain a specimen of blood the needle is inserted into a vein, and the capillary end of the ampule crushed with a hemostat through the rubber tubing, blood flowing into the ampule and replacing the vacuum (Figs. 14 and 15). The protecting glass tubing is then replaced.

Not infrequently, especially in children and in obese adults, one fails to enter a vein. Several attempts to do so may result in ruining one or more of the tubes. The late Dr. Alfred Reginald Allen devised a useful modification in the technic of using this handy tube; this consisting in detaching the



FIG. 20.—METHOD OF OBTAINING BLOOD BY CUPPING. (Keen's Surgery.)

The child is seven years of age; the Blackfan apparatus is being used and blood collected from a scarified area into a sterile test-tube.

bulb from the rubber tubing and needle, inserting the latter into the vein, and when the blood appears, quickly attaching the bulb and breaking the neck with a hemostat, in the usual manner. By this method the bulb is not broken until one is sure he has entered a vein and secured a specimen of blood.

(b) **Wet Cupping.**—1. This method is particularly applicable for securing blood from infants.

2. Cleanse an area over the back just below the angle of the scapula.
3. Scarify with a few superficial linear incisions or with a special scarifier.
4. Apply a cup and exhaust the air with special syringe. The vacuum produces marked congestion of the skin with a ready flow of blood.
5. Carefully release the cup and pour blood into a tube.

6. The apparatus devised by Blackfan, and shown in the accompanying illustrations (Figs. 19 and 20), is quite satisfactory and collects blood in a sterile tube.

(c) **Placental Blood.**—For purposes of immunization corpuscles may be obtained by collecting placental blood.

1. After tying and cutting the cord the placental end is placed carefully in a 150 c.c. flask or bottle containing from 25 to 50 c.c. of sterile 2 per cent. sodium citrate in physiologic salt solution. To avoid contamination the cord may be lightly sponged with 1 per cent. formalin solution and severed with sterile scissors.

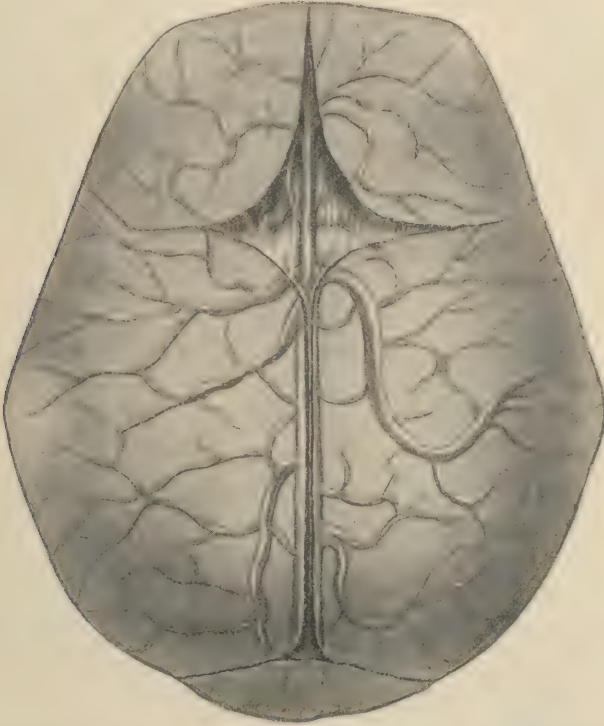


FIG. 21.—THE ANTERIOR FONTANEL AND RELATIONS AND SIZE OF THE SUPERIOR LONGITUDINAL SINUS IN A NEWBORN INFANT. (Keen's Surgery.)

2. By exerting pressure on the uterus blood may be squeezed out of the placenta. The flask is then sealed with a sterile cotton plug and gently shaken.

3. The corpuscles are obtained by centrifugalization or sedimentation.

(d) **From Superior Longitudinal Sinus.**—In *infants* fifteen months or less in age blood is readily obtained from the superior longitudinal sinus if the *anterior fontanel* is still open (Figs. 21 and 22). The latter operation, first conducted by Tobler,¹ and since highly recommended by many pediatricists, is conducted as follows:

The skin is carefully cleansed and sterilized with tincture of iodine; the puncture is best made in the median line of the posterior angle. The needle attached to a 5 c.c. syringe (Luer or Record) should be about gage No. 18

¹ Monatschr. f. Kinderhl., 1915, 13, 384.

with a short sharp bevel, and is passed inward at a right angle for a distance of about 4 mm. and suction made (Fig. 23); if blood does not flow the needle should be passed about 2 mm. further, which suffices for the majority of children up to fifteen months of age. The needle and syringe



FIG. 22.—A CROSS-SECTION OF THE HEAD OF A NEWBORN INFANT THROUGH THE POSTERIOR ANGLE OF THE ANTERIOR FONTANEL TO SHOW THE SIZE AND SHAPE OF THE SUPERIOR LONGITUDINAL SINUS. (Keen's Surgery.)

should be carefully sterilized by boiling and the whole operation conducted in an aseptic manner. With proper care the operation may be done as a safe, quick, and efficient means for obtaining small amounts of blood from infants. Goldbloom¹ has devised a special needle-holder for the purpose



FIG. 23.—METHOD OF OBTAINING BLOOD FROM THE SUPERIOR LONGITUDINAL SINUS AND INJECTING WITH A SYRINGE. (Keen's Surgery.)

The child is six months of age; the shape and size of the anterior fontanel have been outlined; a 5 c.c. Record syringe fitted with No. 18 needle is being employed, the needle having been entered for about 6 mm. in the median line at the posterior angle, and perpendicular to the sinus.

of guarding against passing the needle too far and transfixing the sinus; while it is handy and convenient, the physician accustomed to conducting venipuncture will probably find it unnecessary and prefer to pass a needle

¹ Amer. Jour. Dis. Child., 1918, 16, 388.

attached to a syringe slowly and carefully, making gentle suction with the piston at intervals to determine when the sinus has been entered.

The transfusion of blood, serum, salt solution, and neo-arsphenamin is easily conducted by this technic, the injections being given with a syringe; in injecting arsphenamin and neo-arsphenamin great care must be exercised, as disastrous results have followed faulty technic due to the injection of these irritating substances into the brain.

METHOD OF SECURING CEREBROSPINAL FLUID (RACHICENTESIS)

The chief purpose in making spinal puncture is to obtain and examine cerebrospinal fluid as an aid to the diagnosis of cerebrospinal diseases. It is mainly of value in neurologic and psychiatric practice, for the purpose of securing fluid for making the Wassermann reaction, for a study of cytologic changes, alterations in protein content, and the like. Not infrequently the procedure is required as an aid to establishing a diagnosis of meningeal diseases in children, particularly tuberculous meningitis, epidemic cerebrospinal meningitis, meningeal irritation, "serous meningitis," etc.

Contraindications.—Ordinarily, when skilfully performed, spinal puncture is a harmless procedure. Unless the necessity for obtaining fluid is very urgent the operation should not be done on persons in poor physical condition. Kaplan has cautioned against making lumbar puncture in the presence of tumors of the posterior fossa, particularly of the cerebellum. When it is highly desirable to study the fluid of such cases 2 c.c. may be withdrawn, and immediately replaced with sterile normal salt solution, or if no immediate effects are observed, the patient may be kept in bed for the next twenty-four hours.

The rapid withdrawal of fluid, and especially rapid withdrawal with the patient in an upright position, may create sufficient negative pressure in the brain stem to produce hyperemia, hemorrhage, and foraminal hernia, the engagement of the brain stem in the foramen magnum; hyperemia and reflex disturbances of the choroid plexus may be followed by hypersecretion of fluid with increased intracranial pressure which are probably responsible for the headache, vertigo, and vomiting sometimes following the operation rather than the leakage of fluid into the epidural tissues following withdrawal of the needle. In view of the great number of times lumbar puncture is performed the percentage of accidents and complications, however, are comparatively small and the operation may be done with considerable safety if certain precautions are observed as follows: (1) Have the patient lying on the side rather than sitting; (2) evacuate slowly, preferably measuring the pressure after the escape of every 1 to 2 c.c. when drawing fluid from persons with choked disk, and suspected as suffering with brain tumor; (3) never remove more than 5 c.c. of fluid for diagnostic purposes unless the pressure is high due to increased volume of fluid in meningitis; (4) stop if the patient complains of headache; (5) keep the patient in bed for eighteen to twenty-four hours. In common with many others I have frequently permitted patients to be up and about after withdrawal of spinal fluid without any ill effects, but believe that this practice has been responsible for several instances of lumbar puncture headache, and consider advisable the precaution of routinely keeping the patient in bed for at least eighteen hours.

Preparation of Patient.—In bed-fast patients the puncture may be made at any time; with ambulatory patients, however, the most suitable time is late in the afternoon, so as to permit the patient to rest overnight.

The ordinary preparations consist in scrubbing the skin of the lumbar region with green soap and hot water, using gauze sponges, followed by washing with alcohol and ether. The area is then covered with sterile gauze, and just before the puncture is made an application of 10 per cent. tincture of iodine is made; or the preliminary cleansing may be omitted, two or three coats of the iodine being sufficient. After the fluid has been secured the iodine may be removed with alcohol and gauze. The operator's hands should be cleansed carefully and washed in alcohol and bichlorid solution or weak formalin, or he may put on sterilized rubber gloves before handling the needle and performing the operation itself.

Anesthesia.—In the majority of instances an anesthetic is not necessary. In *tabes dorsalis* and general paralysis (two conditions most frequently



FIG. 24.—METHOD OF PRODUCING LOCAL ANESTHESIA IN SPINAL PUNCTURE. (Keen's Surgery.)

The patient is an obese adult male; the crest of the right ilium is outlined and the "soft spot" between the third and fourth lumbar vertebrae located. An *intracutaneous* injection of sterile 1 per cent. novocain is being made with a 1 c.c. Record syringe fitted with a No. 26 needle. The position of the needle will then be changed to the perpendicular and the subcutaneous tissues infiltrated as far as the needle will reach.

requiring spinal puncture) the operation is peculiarly painless. Sick children are apparently not greatly disturbed, but in adults it may be necessary to infiltrate the skin about the site of puncture with 1 per cent. eucain (sterile) or cocain solution. The skin over the "soft spot" is infiltrated with a fine needle (No. 26), and 1 c.c. syringe, a whitish elevated patch about the size of a dime being produced (Fig. 24); the needle is now slowly passed vertically, and the deeper tissues anesthetized to the depth of the hypodermic needle. After withdrawal the spinal puncture needle is introduced in the same opening. Ethyl chlorid is much less satisfactory except for the mental effect it has upon the patient. Children may receive a few drops of ether. With nervous patients it is good practice to obviate nervous shock by adopting a few simple precautions against causing unnecessary pain.

Measuring the Pressure of Spinal Fluid.—The Landon mercurial man-

ometer is quite useful; finer and more accurate readings, however, are possible with an air or a water manometer, the fluctuations of the column of fluid being greater.

In taking the pressure it is imperative that *a uniform technic be employed*; for example, the patient should be in a quiet horizontal position. Upright posture and coughing increase the pressure.

The pressure is read before fluid is allowed to escape; the position of the instrument is shown in Fig. 25.

The *normal pressure* of cerebrospinal fluid varies greatly according to technic, and readings are of value only when a uniform method is employed. The normal pressure for adults in a quiet horizontal position with mercury manometer of Landon is from 6 to 10 mm., with an average of 8 mm.;

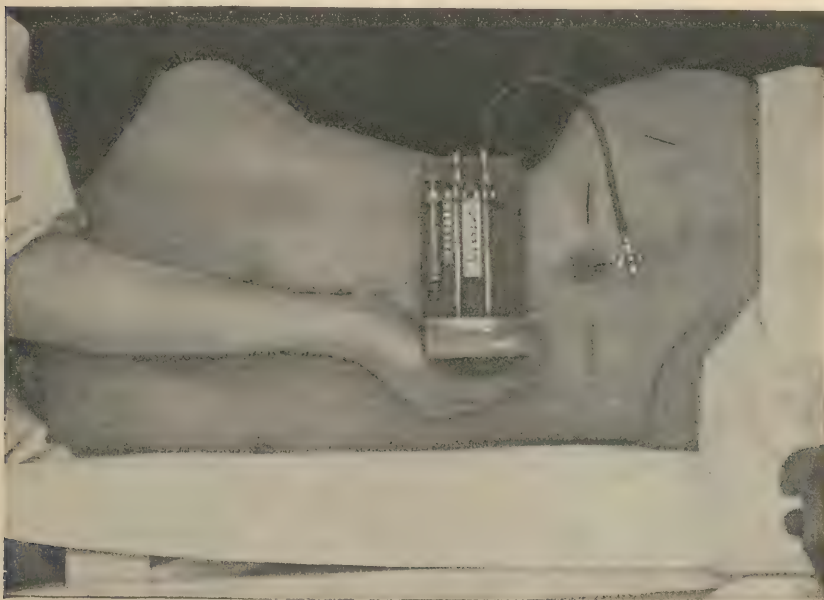


FIG. 25.—SPINAL PUNCTURE IN THE RECUMBENT POSTURE AND MEASUREMENT OF SPINAL FLUID PRESSURE WITH A LANDON MERCURIAL MANOMETER. (From Frazier's *Surgery of the Spine and Spinal Cord*, D. Appleton & Co., New York.)

The special needle is in position and the manometer adjusted for reading of the pressure before spinal fluid is collected; the reading in this instance was 20 mm. of mercury. The instrument is made by the Harvey E. Pierce Co. of Philadelphia.

with the manometer of Levinson, from 130 to 150 mm. The pressure of children is about one-third less, being 45 to 90 mm. of water and 0 to 4 mm. of mercury. Pressure in millimeters of water may be expressed in millimeters of mercury by dividing by 13.

Cerebrospinal fluid pressure may be increased in a variety of conditions: (1) In certain cases of congenital or acquired internal and external hydrocephalus with hypersecretion and normal or impaired absorption; (2) in acute and chronic meningitis; (3) in acute meningeal congestion—the so-called “serous meningitis”; (4) in hemorrhage, and (5) in various space-restricting lesions such as tumors and fragments of bone and localized chronic inflammatory changes.

Technic of Lumbar Puncture.—The patient may either sit in a chair

and bend forward, or lie on the left side on the edge of a bed or table (Fig. 25). In the case of sick persons, particularly children, the latter position is necessary; it is also advisable with nervous patients, as they are likely to bend backward suddenly or jump up when the needle is inserted, and I have known the needle to be broken off at such a time. The back should be arched backward, the patient bending forward, and the knees being drawn up over the abdomen.

With the sitting posture, however, lumbar puncture is an easier operation; the patient may either sit straddling a chair or on the edge of a table or bed with the body well bent forward, and the arms folded over a pillow in the pit of the abdomen (Figs. 26 and 27). The patient should be comfortable, relaxed, and the back well arched to widen the interarticular spaces.

The *needle* used for lumbar puncture should be selected with care and be neither too large nor too small. In my experience only two sizes are



FIG. 26.—SPINAL PUNCTURE IN THE SITTING POSTURE. (Keen's Surgery.)

The patient is an obese adult male, bent well forward over a folded pillow; the crests of the ilia are outlined. The needle has been passed between the third and fourth lumbar vertebrae in the median line; spinal fluid is being collected in sterile graduated centrifuge tubes.

required—one with an outside diameter of 1 mm. (gage No. 19) for the practically painless puncture of persons, and particularly adults, in whom there are no symptoms of infectious meningitis, and the spinal fluids of normal consistency, and a second of larger caliber (gage No. 15) for use when suppurative meningitis is suspected, in which case the spinal fluid may be denser and flow less easily (Fig. 28). For infants both needles may be cut in half and properly pointed, although these smaller sizes are not absolutely necessary. Needles are available made of platinum and steel; the former bend very easily when brought in contact with bone and thus protect the patient, but likewise are easily bent in the operation, and deflected from the proper course by muscular movements, and for these reasons I prefer the latter. It is important for the needle to have a short beveled tip and not a very sharp point.

The needle should be sterilized by boiling in water for several minutes.

The operator now selects a "soft spot" for puncture. By running the finger along the spines of the vertebræ this will be found to be between the third and fourth lumbar spinous processes, about on a level with the posterior superior spines of the ilia. The needle is grasped firmly and inserted with a sudden thrust, exactly in the median line, and straight forward. The thrust should be sufficient to push the needle through the skin and muscles into the spinous ligaments; it may then be inserted more slowly, a sudden "give way" indicating that the canal has been entered (Fig. 29). This route is better than the lateral route, as there is less danger of striking vertebral processes or other obstructions. The stilet is now withdrawn.



FIG. 27.—TECHNIC OF SPINAL PUNCTURE.

The patient is sitting on the edge of a chair and is bent forward; the crests of the ilia are indicated by black lines, and are on a level with the spinous process of the fourth lumbar vertebra; the "soft spot" is found just above. The needle is shown in Fig. 28. The first tube receives the first few drops of fluid, which are usually blood tinged.

Usually the first fluid to appear is stained with blood and should be collected in a separate tube. From 5 to 10 c.c. of fluid are then collected in a second sterile tube, the needle is quickly withdrawn, and the puncture wound sealed with collodion and cotton or with adhesive plaster.

It sometimes happens that, on withdrawing the stilet, no fluid issues forth. In this case the patient is instructed to take a deep breath, and if fluid does not appear now, the stilet may be inserted gently to dislodge any material that may be occluding the needle, or the needle may be withdrawn a trifle if it has been inserted too far, or may be advanced a little if it has not entered the canal. If, however, the tap proves a dry one, or if only a few drops of blood are obtained, it is not advisable to make another

puncture, as the second attempt is likely to prove as unsuccessful as the first.

After-treatment of the Patient.—Occasionally the needle may strike a nerve filament, which occurrence is followed by more or less pain along the course of its distribution; puncture of the bone is likely to be followed by pain for several hours. The majority of patients are so little affected by lumbar puncture that no precautions as regards the after-treatment are necessary. As previously stated, it is advisable for the patient to rest overnight. Sudden release of pressure or the nervous shock may give rise to severe headache of one or of several days' duration; persons of hysteric temperament may, in addition, suffer from diarrhea and vomiting. Rest in bed, the application of ice-bags, and the administration of sedatives are usually sufficient to relieve these after-effects.

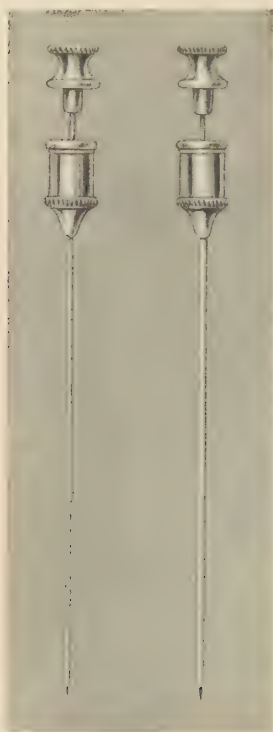


FIG. 28.—BARCOCK NEEDLES FOR SPINAL PUNCTURE. (Keen's Surgery.)

The needle on the left is gage No. 19 with an inside diameter of 1 mm., and recommended for routine spinal puncture and intraspinal injections when the spinal fluid is of normal consistency. The needle on the right is gage No. 15 with an outside diameter of 1.75 mm., and recommended for spinal puncture and intraspinal injections in suppurative meningitis with thickened spinal fluid. Both needles fit the various sizes of Record syringes.



FIG. 29.—AN IMPROPERLY CONSTRUCTED NEEDLE WITH THE OPENING SO LONG AND BEVELED THAT HALF OF THE OPENING MAY BE WITHIN THE DURAL SAC AND HALF WITHOUT, ALLOWING ESCAPE OF FLUID INTO THE EPIDURAL SPACE. (From Frazier's Surgery of the Spine and Spinal Cord, D. Appleton & Co., New York.)

Disposal of the Fluid.—As a general rule, the fluid should be sent at once to a laboratory, as total cell counts and bacteriologic cultures are best made with fresh fluid. For the Wassermann reaction it is not advisable or necessary to add a preservative, as the fluid will keep for several days in a good refrigerator; if, however, the fluid is to be kept for longer periods of time, 0.1 c.c. of a 1 per cent. solution of phenol may be added to each

cubic centimeter of fluid. The chart shown in Fig. 30 has been found quite useful for recording the results of examination.

CEREBROSPINAL FLUID EXAMINATION												
Name:		Age:		Ward:		Physician:		Date:				
Clinical Diagnosis:												
Pressure	Amount	Physical Properties	Cells per c. mm.	Differential Cell Count			Protein Tests			Wassermann Reaction		
				Lymph.	Polys.	Endothel.	Pandy	Noguchi	Kaplan	Blood	Spinal Fluid	
Colloidal Gold Reaction												
Registry No.	Color Reactions	Dilutions of Spinal Fluid										Remarks
		1 1:10	2 1:20	3 1:40	4 1:80	5 1:160	6 1:320	7 1:640	8 1:1280	9 1:2560	10 1:5120	
5	Colorless.....											
4	Pale blue.....											
3	Blue.....											
2	Light or purple.....											
1	Red-blue.....											
0	Red—unchanged.....											
Bacteriological Examination Culture: _____ Animal Inoculation: _____ Additional Chemical Examinations Quantitative Protein: _____ Quantitative Chloride: _____ Examined by: _____												

FIG. 30.—A CHART USED BY THE AUTHOR FOR RECORDING THE RESULTS OF EXAMINATIONS OF CEREBROSPINAL FLUID. (Keen's Surgery.)

OBTAINING SMALL AMOUNTS OF ANIMAL BLOOD

Rabbit.—1. Flip an ear vigorously with the hand, and rub with xylol and alcohol. The xylol produces marked congestion and afterward should be carefully removed with alcohol and water, as it produces a low-grade inflammatory reaction.

2. Puncture a marginal vein with a large needle. The blood will flow quickly in drops and practically any amount up to 10 c.c. or even more may be collected in a centrifuge or test-tube (Fig. 31). For making preliminary tests of serum during immunization 2 c.c. of blood is usually sufficient. Bleeding may be checked by making firm pressure over the puncture.

Guinea-pig.—1. Blood may readily be removed directly from the heart by anesthetizing the animal with ether, and inserting a sterile needle into the heart at the point of maximum pulsation. A syringe for aspiration

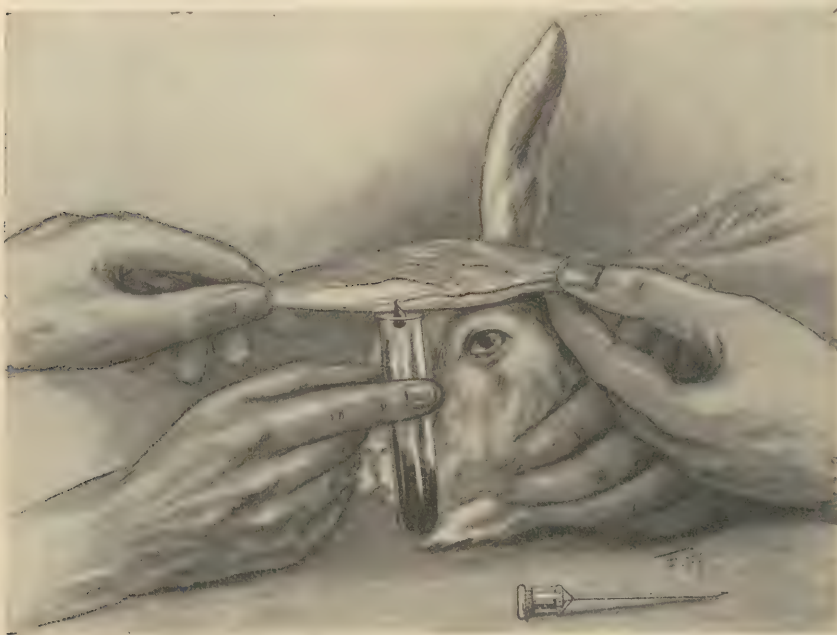


FIG. 31.—METHOD OF BLEEDING A RABBIT FROM THE EAR.

may be attached, but better results are secured by adjusting a suction apparatus. By means of a short piece of rubber tubing the needle may be connected to a test-tube so arranged that a partial vacuum is created by attaching to a water suction pump. As soon as the heart has been entered, blood is seen to flow into the tube and the constant suction prevents clot formation in the needle. In this manner 5 c.c. of blood may readily be obtained.

2. Blood may also be secured by aspirating the external jugular vein. The vein is exposed by making a small incision, as in giving intravenous injections.

3. Sufficient blood to make many complement-fixation tests may be secured from a large pig by rubbing the ear vigorously with xylol and making a small incision in the margin. Bleeding is facilitated by attaching a

small test-tube with a side arm to a suction pump. When the proper tube is held firmly over the ear 5 c.c. of blood may be obtained by this method.

Sheep.—Small amounts of blood may be obtained by puncturing one of the ear veins.

OBTAINING LARGE AMOUNTS OF ANIMAL BLOOD

Rabbit.—After immunization of a rabbit has been completed the animal is usually bled to death, the object being to secure the maximum quantity of serum in a sterile condition. Various methods may be used. The animal

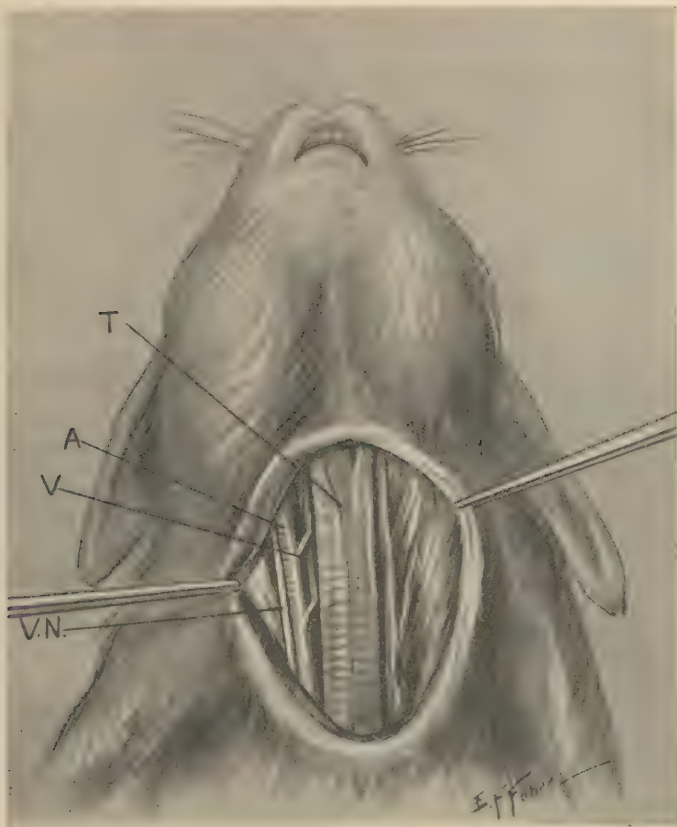


FIG. 32.—A DISSECTION OF THE NECK OF A RABBIT TO SHOW THE RELATIONS OF THE CAROTID ARTERY.
T, trachea; A, carotid artery; V, internal jugular vein; V.N., vagus nerve.

should be anesthetized by ether or high rectal injection of a gram of chloral hydrate in 10 c.c. of water, deep sleep being induced by the latter in from five to ten minutes, and lasting for several hours, during which time operative procedures produce no pain.

First Method (Nuttall).—The animal is fastened to an operating board or, preferably, held by an assistant, and the hair over the neck and thorax is moistened with a 1 per cent. lysol solution. By means of a sterile knife the skin is cut longitudinally and the neck muscles exposed for a considerable distance. The animal is then held upright by the assistant over a sterile dish or a large sterile funnel, emptying into a cylinder or 50 c.c.

centrifuge tube. The operator stretches the neck by carrying the head backward, and severs the large vessels on one or both sides of the neck with a sharp sterile scalpel or razor, avoiding opening the trachea and esophagus. After bleeding, the dish is covered or the tube plugged and set aside for the serum to separate. This method is quite simple, may be employed by the inexperienced, and usually yields a large amount of sterile serum.

Second Method.—The animal is fastened to the operating board and the neck is stretched by placing a roller beneath it. The hair over the neck is clipped close, and the skin moistened with alcohol and 1 per cent. lysol solution. The carotid artery of one side is exposed by making a straight incision through the skin over the trachea and skinning well to one side,



FIG. 33.—METHOD OF BLEEDING A RABBIT FROM THE CAROTID ARTERY, SECOND METHOD.

exposing the sternohyoid muscles and external jugular vein. The carotid artery, internal jugular vein, and pneumogastric nerve are to be found at the outer border of the sternohyoid muscles (Fig. 32). By means of blunt dissection the artery is exposed and carefully isolated. Two small spring clamps or hemostats are then applied close together at the distal end, and the artery divided between them. The proximal end is then held with forceps within the mouth of a sterile cylinder or large centrifuge tube. The wall of the artery is incised with fine scissors proximal to the forceps, and the blood is allowed to flow into the vessel. The yield of blood may be increased somewhat by exerting pressure on the animal's abdomen and thorax.

To avoid the risk of contamination in the foregoing method the apparatus shown in Fig. 33 may be used. The whole apparatus is sterilized in the autoclave before using. After the artery has been exposed and isolated a temporary clamp is applied to the proximal end. A small incision is made in the wall of the artery, and the cannula inserted and fastened with a ligature. The clamp is then removed and blood collected in a large tube.

Third Method.—The following method, employed at the Pasteur Institute at Paris, has been found very useful. The animal—a rabbit or a guinea-pig—is anesthetized and secured to an operating-table. The carotid artery is carefully and aseptically exposed, and separated from the tissues for a distance of at least 1 inch; a ligature is now tied securely about the artery at the distal end of exposure; a second ligature is placed in position and looped loosely, ready to tie about the proximal end (Fig. 34).



FIG. 34.—METHOD OF BLEEDING A RABBIT FROM THE CAROTID ARTERY, THIRD METHOD.

The bottom of a large sterile test-tube is heated and drawn out to a fine point, as shown in the illustration (Fig. 34), and the tip is broken off. The operator now places his moistened forefinger under the artery, elevating and rendering it taut; the tip of the tube is then passed through the wall into the interior of the vessel toward the heart. The moment the vessel is entered the blood-pressure drives the blood into the tube, so that 20 c.c. are soon secured. An assistant now ties the ligature below the site of puncture; the tube is withdrawn, and the tip sealed in a flame. The ends of the ligatures are cut short and the wound is stitched. Healing usually occurs at once, and if subsequent study of the blood is required, the other carotid and the femorals can be used similarly for securing it.

Fourth Method.—The animal is fastened to the operating board, and

the hair over the neck and thorax moistened with alcohol or lysol solution. The right thorax is then incised and held open by an assistant. The right lung is seized with sterile forceps and quickly severed at the base with



FIG. 35.—METHOD OF BLEEDING A GUINEA-PIG.

sterile scalpel or scissors. The heart is then punctured, and the blood is quickly removed from the thoracic cavity with a sterile 25 c.c. pipet with a large opening. Unless the lung is removed it tends to float and block the

end of the pipet. Everything must be in readiness, as otherwise blood will be lost, flooding the thoracic cavity.

Guinea-pig.—Pig serum is usually secured to furnish complement in hemolytic tests, and should be used within twenty-four or forty-eight hours after bleeding. Precautions to insure sterility are not, therefore, usually necessary.

1. The animal is anesthetized with ether and the large vessels of the neck on one side are exposed by a longitudinal incision. These are severed, and the blood is collected in a Petri dish or in a centrifuge tube by means of a funnel (Fig. 35).

2. By means of a sharp-pointed scissors the vessels on one or both sides of the neck may be incised transversely at one cut, inserting the blade deeply and close to, but avoiding, the trachea and esophagus.

3. Small amounts of blood may be obtained by aspiration from the heart of the living animal. A 5 c.c. Record syringe fitted with a No. 20 or



FIG. 36.—METHOD OF SECURING BLOOD FROM HEART OF A GUINEA-PIG.

22 needle is employed. The animal is fastened to a board or held by an assistant and lightly anesthetized. The point of maximum pulsation is determined and the needle slowly entered into the right chambers of the heart. As a general rule, 2 to 5 c.c. of blood may be obtained by gentle suction, the amount depending upon the size of the animal. Large male animals are recommended and may be used repeatedly. After withdrawal of the needle the animal rapidly recovers, although occasionally bleeding may follow into the pericardial sac (Fig. 36).

Rats.—1. Small quantities of blood may be obtained by snipping off the tip of the tail of the animal and milking blood into an appropriate sterilized tube containing glass beads, or 2 per cent. sodium citrate solution. In this manner one or more cubic centimeters of blood are easily obtained, and at once defibrinated and injected into the peritoneal cavities of other animals, as in inoculating trypanosomes, etc.

2. Large quantities of blood are obtained by severing the large vessels of the neck under anesthesia.

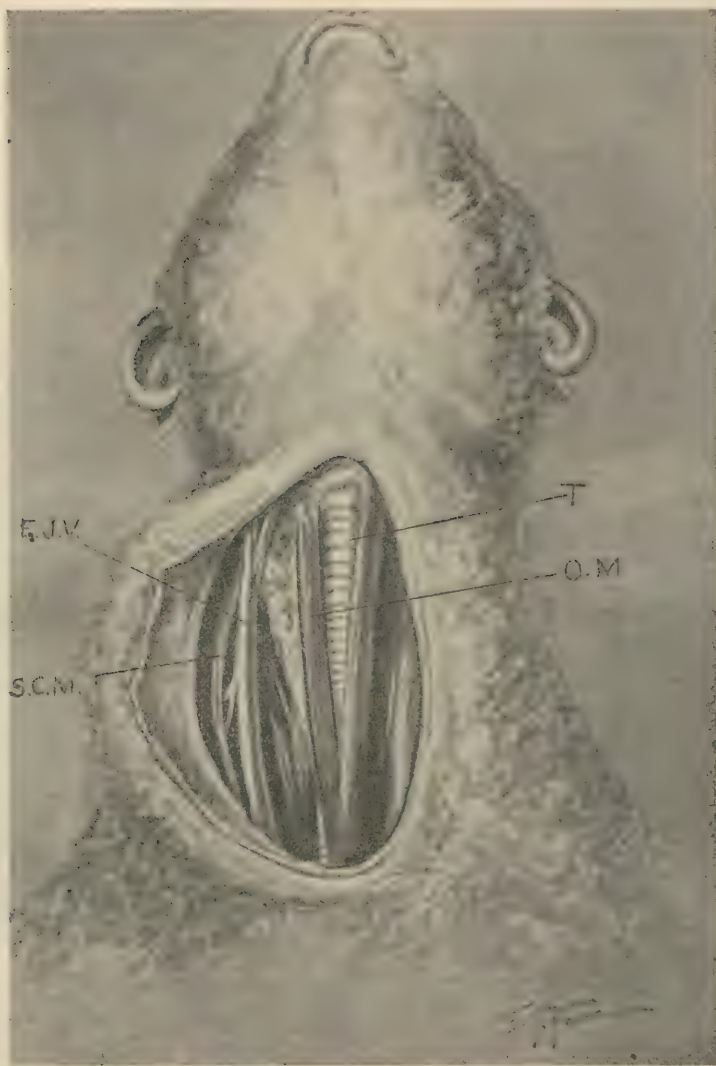


FIG. 37.—A DISSECTION OF THE NECK OF A SHEEP TO SHOW THE RELATIONS OF THE EXTERNAL JUGULAR VEIN.

T, trachea; *O.M.*, omohyoid muscle; *E.J.V.*, external jugular vein; *S.C.M.*, sternocleidomastoid muscle. This dissection was made soon after natural death and shows the position and size of the vein with the head held backward as it is when blood is removed according to the technic described in the text. When distended the vein is even larger than shown; it is quite superficial and is usually palpable when pressure is made over the vein at the base of the neck.

Sheep.—Blood may easily be obtained from a freshly killed animal. The first flow of blood is discarded, and a portion of the remainder is collected in a large, sterile, thick-walled flask containing glass beads. By shaking vigorously the blood is defibrinated if one desires to obtain cor-

puscles, or the blood may be collected in a cylinder and defibrinated by whipping with glass rods.

It is usual, however, in large laboratories to keep a sheep and remove the blood as it may be required. Small amounts may be obtained from the ear vein, larger quantities being secured from an external jugular vein in the following manner:

1. One may do the bleeding alone, although the aid of an assistant is usually necessary, especially if the animal is large and vicious.

2. The sheep is thrown on its back, and the head is held on the knees of an assistant seated on a low box or stool.



FIG. 38.—METHOD OF BLEEDING A SHEEP FROM THE EXTERNAL JUGULAR VEIN.

The operator is distending the vein by pressure over the base of the neck with the left hand. When distended the vein can usually be felt beneath the skin. The needle here shown is reduced to a little more than half the actual size.

3. The operator may straddle the animal to hold down the fore feet, although this is not necessary unless the animal is vicious.

4. The wool on the left side of the neck is clipped closely with scissors and alcohol applied.

5. The operator then grasps the neck low down with the left hand, and by means of the thumb exerts pressure over the base of the neck. The external jugular vein will be found in a groove between the omohyoid and sternomastoid muscles (Fig. 37). Firm pressure over the base of the neck usually distends the vein, which may be seen or easily felt. After locating the vein the pressure should be released for an instant, when the disten-

tion will disappear. In this way the operator may be more certain that he has located the vein.

6. A sterile stout needle, at least 2 inches in length and provided with a trocar and special shank for firm grasping, is passed quickly into the distended vein in an upward and inward direction (Fig. 38). It is essential that the needle be sharp, otherwise it will be turned aside by the wall of the vein. The end of the needle must not have too long a bevel, or the point will pierce the opposite wall before the body of the needle is well within the vein. The trocar is now removed, and blood collected in a flask or bottle and defibrinated with glass beads and rods. A short piece of rubber tubing may be attached to the needle. A suction apparatus is not needed because the flow of blood is good so long as pressure is preserved over the vein at the base of the neck.

7. When the required amount of blood has been secured, pressure is released and the needle quickly withdrawn. Bleeding ceases at once, and the neck is then washed with alcohol.

8. By this method the same vein may be used over and over again for several years. I have never known infection to occur, although the gradual formation of scar tissue about the site of puncture may interfere with the operation.

Hog.—Blood may be secured from hogs by clipping off a small portion of the tail with a sharp razor or scissors, beginning at the tip. Bleeding is usually quite free, but is easily controlled by a tourniquet and bandage. The serum of hogs immunized against hog cholera is secured in this manner.

Monkey.—1. Small quantities of blood—up to 10 or 20 c.c.—may readily be obtained from a small vein just beneath the skin which crosses over the inner malleolus at the ankle. When a tourniquet is applied the vein becomes prominent; the hair is clipped, and tincture of iodine applied over the skin; a small needle is passed into the vein, and the blood collected in a centrifuge tube.

2. Large quantities of blood may be obtained from the femoral or external jugular vein under light ether anesthesia.

Dog.—1. Small quantities of blood may be obtained in the following manner: Apply a tourniquet just above the knee; clip the hair over the anterior surface of the leg, and cleanse with tincture of iodine and alcohol; make a small incision in the long axis, exactly in the median line; a fairly large vein appears at once just beneath the skin; by inserting an appropriately sized needle several cubic centimeters of blood are quickly and easily secured. The wound should be very small, and usually requires no treatment other than an application of collodion and cotton.

2. Large quantities of blood are obtained from the external jugular vein with or without ether anesthesia; the neck is shaved and cleansed; the skin is incised over the vein, which is just beneath the skin, and blood removed with a sterile needle and syringe. Pressure over the base of the neck renders the vein more prominent. In the case of large dogs incision is not necessary, as it is easy to enter the vein directly through the skin, as in bleeding the sheep from the external jugular vein or the human from a vein at the elbow. Blood may also be secured from the femoral vein under ether anesthesia.

Horse.—1. Small quantities of blood for making agglutination and complement-fixation tests may readily be secured from a superficial vein about the leg. The hair is clipped over the selected area, and the skin sterilized with tincture of iodine. A tourniquet is applied to render the vein prominent, the vessel is steadied between forefinger and thumb, and a needle quickly inserted.

2. Larger quantities of blood are secured from the external jugular vein. This operation is easily conducted in an aseptic manner and blood collected in sterile jars. The neck about the region of the vein, usually on the left side, is clipped, and a large area washed with hot lysol solution. A sterile sheet may be thrown about the shoulders. The animal is held or placed in specially constructed stalls that prevent him from backing away or causing mischief. In large antitoxin laboratories bleeding is conducted in special rooms, where a careful aseptic operating-room technic may be observed.



FIG. 39.—BLEEDING A HORSE FROM THE JUGULAR VEIN.

The external jugular vein is rendered prominent by exerting pressure at the base of the neck by the application of a special tourniquet or by the thumb and fingers of the left hand, the thumb being placed just above the vein. A small incision is made through the skin directly above the vessel.

A large needle is passed under the skin for a distance of an inch or two and then thrust into the vein. Direct puncture into the vein is avoided, as the needle track under the skin closes after the needle is withdrawn and serves to seal the puncture. The needle is attached to sterile rubber tubing that conducts the blood into special jars (Fig. 39). In this manner from 6 to 12 liters of blood are easily obtained.

CHAPTER III

TECHNIC OF ANIMAL INOCULATION

THIS is a highly important part of immunologic work, as both for serum diagnosis and for serum therapy the serum must be secured from animals that have been artificially immunized. Successful inoculation requires unremitting care and thoroughness, as the toxic effects of the proteins in general may kill an animal before immunization has been completed. No hard-and-fast rules can be laid down; the weight of an animal and the reaction to an injection should decide the frequency and the size of subsequent injections. It is better to proceed slowly and gradually than to give too large a dose at once and at too frequent intervals.

GENERAL RULES

1. Select an appropriately sized syringe that does not leak upon being tested with water. As has been stated elsewhere, nothing is more unsatisfactory than a leaking syringe, for not only may the hand become soiled, but an unknown quantity of inoculum is lost.

2. The inoculum should be sterile. This is especially desirable when giving intravenous and intraperitoneal injections. When living cultures of bacteria are to be injected the syringe and the needle should be sterilized in order to avoid the introduction of contaminating organisms.

3. Remove the plunger from the barrel, and sterilize all the parts by boiling for at least one minute. As previously stated, an all-glass syringe or a glass barrel and metal plunger is the most satisfactory (see Fig. 7). The old-fashioned syringe with washers and rubber-tipped plunger should find no place in a laboratory.

4. After cooling, expel the water and load the syringe. This may be done by drawing the fluid directly into the syringe and measuring the dose by its markings or by pipeting the exact dose into a sterile Petri dish or capsule and drawing up in the syringe.

5. The animal should be fastened or held firmly and in an easy position. Everything should be in readiness, so that the injections may be given thoroughly and carefully.

6. In preparing the inoculum care should be exercised that no solid particles enter the syringe. Aside from possibly blocking the needle and interfering with the injection, the subcutaneous injection of small fragments may do no particular harm, but in intravenous inoculation they may cause fatal embolism. To obviate this danger the inoculum should, if possible, be filtered through sterile filter-paper before the syringe is filled.

7. Air-bubbles should be removed. The injection of small bubbles of air into subcutaneous tissues may cause no harm, but when injected into veins they may cause serious disturbances or immediate death. To avoid this the syringe, after being filled, should be held vertically, with the needle uppermost. The needle should be wrapped in cotton soaked in alcohol, and the piston of the syringe pressed upward until all the air is expelled from the barrel and the needle. If a drop of inoculum is forced out, it will be collected on the cotton, which should immediately be burned.

8. Injections should be given slowly.

9. The animal is then tagged or marked, or its coloring recorded. In the case of rabbits, the metal ear tag is best. All data, *e. g.*, the date, size of dose, preparation and kind of inoculum, etc., should be recorded in writing.

10. When it is necessary to incise the skin in order to reach a vein an anesthetic may be given. With superficial veins, and in subcutaneous inoculations, the injections may be given so readily and easily that no more pain can be felt than that which accompanies similar injections in human beings.

Animals may be actively immunized in a variety of ways and in different locations in the animal body. For a particular antibody a certain method may be found especially efficacious, and this is dealt with in a subsequent chapter. In serologic work immunization may be performed by *subcutaneous*, *intramuscular*, *intravenous*, *intracardial*, and *intra-peritoneal* injections.

METHOD OF PERFORMING SUBCUTANEOUS INOCULATION

Fluid Inoculum.—1. Injections are usually given in the median line of the abdominal wall.



FIG. 40.—SUBCUTANEOUS INOCULATION OF A GUINEA-PIG.

A fold of skin is pinched up and the needle entered into the fold. The skin is then released, and the injection slowly given. A swelling indicates that the injection is subcutaneous.

2. Have the animal (a rabbit or a guinea-pig) held firmly by an assistant or secured to the operating-table.

3. Clip the hair where injection is to be made—it is not always necessary to shave the area. Apply a 2 per cent. iodine in alcohol solution.

4. Pinch up a fold of skin between the forefinger and the thumb of the left hand; hold the syringe in the right hand, and insert the needle into the ridge of skin between the finger and thumb, and push it steadily onward until the needle has been inserted about an inch (Fig. 40). Care must be exercised not to enter the peritoneal cavity. Relax the grasp of the left hand and slowly inject the fluid. If the skin is raised, this shows that the injection is subcutaneous. If it is not, the needle should be slightly withdrawn and inserted.

5. Withdraw the needle, and at the same time cover the puncture with a wad of cotton wet with alcohol. A touch of flexible collodion over the puncture completes the operation.

Solid Inoculum.—Steps 1 to 3 are the same as in the preceding.

4. Raise a small fold of skin with a pair of forceps, and make a tiny incision through the skin with a pair of sharp-pointed scissors.

5. With a probe, separate the skin from the underlying muscles to form a funnel-shaped pocket.

6. By means of a fine-pointed forceps or a glass tube syringe introduce the inoculum into this pocket and deposit it as far as possible from the point of entrance of the instrument.

7. Close the wound with collodion and cotton. A single stitch with fine thread may be necessary.

METHOD OF MAKING INTRAMUSCULAR INOCULATION

1. These injections are usually made into the posterior muscles of the thigh or into the lateral thoracic or abdominal muscles.

2. Clip away the hair over the selected area, cleanse, etc., as for subcutaneous injection.

3. Steady the skin over the selected muscles with the slightly separated left forefinger and thumb.

4. Thrust the needle of the syringe quickly into the muscular tissue and slowly inject the fluid.

METHOD OF MAKING INTRAVENOUS INOCULATION

Rabbit.—1. The posterior auricular vein along the outer margin of the ear is better adapted than a median vein for this purpose.

2. If a number of injections are to be made commence as near the tip of the ear as possible, as the vein may become occluded with thrombi, and subsequent inoculations may then be given nearer and nearer the root of the ear.

3. The animal should be held firmly, as the slightest movement may result in piercing the vein through and through and require reinsertion of the needle. This is accomplished satisfactorily by placing the rabbit upon the edge of the table and holding it firmly there by grasping the neck and front quarters, the assistant at the same time compressing the root of the ear with the thumb and forefinger.

4. If the hair is long, clip it.

5. The ear is struck gently with the fingers and washed with alcohol and xylol; the friction will render the vein more conspicuous.

6. The ear is grasped at its tip and stretched toward the operator, or the vein may be steadied by rolling the ear gently over the left index-finger and holding it between the finger and thumb.

7. The inoculum should be free from solid particles and all the air excluded from the syringe. As a general rule, the amount injected should be as small as possible, and the temperature of the inoculum be near that of the body. If the syringe is filled shortly after sterilization, when it has cooled enough to be comfortably hot to the touch, the heat will warm the injection fluid and not be hot enough to cause coagulation.



FIG. 41.—INTRAVENOUS INOCULATION OF A RABBIT.

8. Hold the syringe as one would hold a pen, and thrust the point of the needle through the skin and the wall of the vein until it enters the lumen of the vein (Fig. 41).

The wooden box shown in Fig. 42 is very convenient for holding rabbits for intravenous injection or for bleeding from the ears.

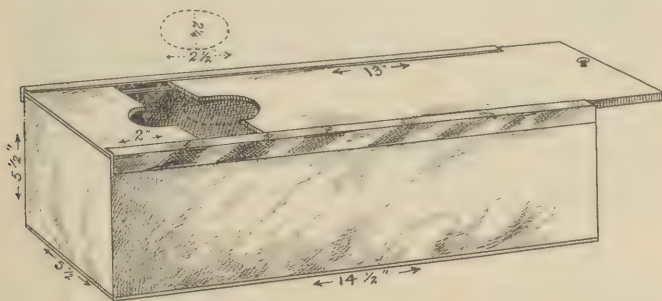


FIG. 42.—A WOODEN BOX FOR RABBITS.

A convenient means for holding the animal for intravenous injection and for bleeding from an ear vein.

9. Direct the assistant to release the pressure at the root of the ear, and *slowly* inject the inoculum. If the fluid is being forced into the subcutaneous tissue, which will be evident at once by the swelling which occurs, the injection must cease and another attempt be made.

10. The needle is quickly withdrawn, a small piece of cotton moistened with alcohol placed upon the puncture wound, and firm compression applied.

Wash the ear thoroughly with alcohol and water to remove xylol, otherwise a low-grade inflammation will follow, which will render subsequent injections more difficult.

Guinea-pig.—1. Since the superficial veins are quite small, it is necessary to make the injection into the external jugular vein. Rous,¹ however, has devised a method for injecting by means of ear veins, which is especially useful for large light skinned animals when repeated injections are to be given. Roth² has described a method utilizing a large superficial vein running diagonally across the back of the hind leg (Fig. 43).

2. The animal is tied to the operating-table and the hair clipped away about the neck and shoulder on the right side, and 2 per cent. iodine in alcohol applied.

3. A small roll is placed under the neck of the animal to render the operative area tenser and more easily accessible.

4. A few drops of ether may be given by an assistant, although one soon learns to expose the vein quickly and there is practically no pain after the



FIG. 43.—ROTH'S METHOD OF INJECTING A GUINEA-PIG INTRAVENOUSLY.

skin has been incised. If anesthesia is employed it should be just sufficient to overcome the struggles of the animal.

5. The assistant is directed to hold the head backward in the median line.

6. Pick up the skin just above and in the middle of the space between the shoulder and the tip of the upper end of the sternum—just above and about in the center of the area where a clavicle in the human would be situated. With small sharp scissors incise the skin for about $\frac{1}{2}$ inch. Separate the subcutaneous tissue gently with forceps; a large vein at once comes into view (Fig. 44). Gently dissect it free for about $\frac{1}{4}$ inch.

7. Pick up the vein with a pair of fine forceps, insert the needle of the syringe gently in the long axis of the vein, and slowly inject the fluid (Fig. 45).

8. Withdraw the needle and apply firm pressure with a wad of clean

¹ Jour. Exper. Med., 1918, 27, 459.

² Jour. Bacteriology, 1921, 6, 249.

gauze or cotton. It is not necessary to tie off the vein. A stitch may be inserted to close the skin wound and flexible collodion applied.

Mice and Rats.—1. Mice and rats may be injected through a caudal vein of the tail. These veins are quite small, and the injection requires a fine needle and some experience in the manipulations.



FIG. 44.—A DISSECTION OF THE NECK OF A GUINEA-PIG TO SHOW THE RELATIONS OF THE EXTERNAL JUGULAR VEIN.

The skin has been turned aside and the superficial fascia and fat removed; the position of the vein is well shown and is readily exposed by a small and superficial incision.

2. Fasten the mouse in a special trap so that the tail alone will be exposed. Grasp the tip between the left thumb and index-finger and hold the tail fully extended.

3. A caudal vein is rendered prominent by the gentle application of heat in the form of hot water, or by vigorous rubbing with xylol or alcohol. The superficial cells become softened, and may be scraped off with a sharp scalpel, exposing a vein on each side of the middle line of the tail.

4. It is usually advisable to have an assistant steady the tail while the

inoculation is being given; a fine needle is essential. Inoculation should begin as near the tip of the tail as possible, and in subsequent inoculations gradually approach the root (Fig. 46).

5. Rats may also be injected through the external jugular vein in exactly the same manner as a guinea-pig is inoculated (Fig. 45). The animal is fastened to a small operating board and an assistant holds the head to the left, which stretches the tissues of the right shoulder and side of the neck. A small incision is made midway between the middle line of the neck and the tip of the fore-shoulder. With superficial dissection a promi-



FIG. 45.—INTRAVENOUS INOCULATION OF A GUINEA-PIG.

The vein is steadied by a pair of fine forceps and the injection given through a small needle. The incision here shown is larger than actually required in practice; the vein is also smaller than normal, as the animal was dead for a few hours prior to making the illustration.

nent vein appears; this vein is picked up with fine forceps and the injection is readily given through a fine needle.

Horse.—1. Horses are usually injected in the external jugular vein.

2. The hair of the neck in the region of the site of inoculation should be clipped and thoroughly scrubbed with a hot solution of lysol.

3. The horse should be held by an assistant; if the animal is vicious the injections should be given in a specially constructed stall.

4. The vein is distended by the operator, who grasps the region with his left hand, the thumb being directly over the vein (Fig. 47).

5. The needle is inserted beneath the skin and passed upward for a short distance, and then thrust into the vein.

6. After the injection has been given either with a syringe or, when the inoculum is large in amount, by gravity from a large jar the needle is quickly withdrawn. Bleeding ceases as soon as pressure over the vein is removed.

Sheep and Goats.—In sheep and goats the intravenous injection is made in the external jugular vein directly through the skin. The hair



FIG. 46.—METHOD OF INTRAVENOUS INOCULATION OF A RAT.

The hairs and superficial layers of the skin have been scraped away with a scalpel. The vein on each side of the middle line appears as a bluish line in the subcutaneous tissues.

is clipped and the part shaved and disinfected. Compression by the finger at the root of the neck renders the vein more prominent. Injections are also readily given through a popliteal or a femoral vein. If necessary, a small incision may be made through the skin in order to expose the vein chosen for the injection.

Dog.—Dogs may be injected through the external jugular or popliteal veins. The animal should be fastened to the operating-table.

2. There is a small vein just beneath the skin in the median line, along the anterior surface of the leg, which is readily accessible. Clip away the hair and disinfect with iodine and alcohol. Direct the assistant to grasp the thigh just above the knee to distend the vein and prevent movement, and make a small incision directly in the median line. A small vein is seen at once. Dissect free or pick up gently with fine forceps and insert a small sharp needle. The injection can thus be readily given. Withdraw the



FIG. 47.—INTRAVENOUS INOCULATION OF HORSE.

The operator causes the vein to distend and become prominent by pressure with the left hand. The needle is entered beneath the skin and is pushed upward for an inch or more before the vein is entered. When withdrawn, this tunneled passage closes and prevents bleeding. Larger injections may be given in the same manner by gravity.

needle, apply firm pressure, and insert a single stitch. Bind the wound with a few turns of a gauze bandage or seal with collodion and cotton.

METHOD OF MAKING INTRACARDIAL INOCULATION

1. Guinea-pigs may be injected by the intracardial route instead of intravenously. The technic is not, as a rule, more difficult, and no ill effects are noticed. Not infrequently, however, attempts to inject in the heart fail, and frequent trials are not permissible on account of the danger of injuring the organ.

2. The animal is tied to the operating board, or held firmly by an assistant; an anesthetic may be given

3. Determine the point of maximum pulsation to the left of the sternum by palpation, and quickly insert a thin, sharp needle at the selected area.



FIG. 48.—METHOD OF PERFORMING INTRAPERITONEAL INOCULATION OF A RABBIT.

The head is held downward; the intestines gravitate toward the diaphragm (note distention); this leaves an area between the umbilicus and pelvis relatively free of intestines and lessens the danger of puncturing the intestines.

A flow of blood indicates that the needle has entered the heart. Attach the previously filled syringe and slowly inject the contents.

4. Detach the syringe in order to make sure that the injection was

intracardial as intended, which is indicated by a flow of blood; then quickly withdraw the needle. The puncture wound may be sealed with collodion.

METHOD OF MAKING INTRAPERITONEAL INOCULATION

Rabbit.—1. Clip the hair and shave an area about 2 inches in diameter in the median abdominal line just below the umbilicus. Apply 2 per cent. iodine in alcohol.

2. Direct an assistant to hold the animal firmly, head down. With the animal in this position the loops of intestine tend to sink toward the diaphragm, leaving an area above the bladder which is sometimes free of intestines (Fig. 48).

3. The syringe is grasped firmly and the needle inserted beneath the skin for a short distance in the direction of the head in the long axis of the animal, when the hand is raised and the needle forced forward through the peritoneum. When the peritoneum has been entered this is evidenced by a relaxation of the abdominal muscles. The needle is then withdrawn slightly and the injection made.

Guinea-pig.—1. Direct an assistant to hold the animal firmly upon its back. This is better than fastening it to an operating-table, for it permits relaxation of the abdominal wall when the injection is to be made.

2. Clip the hair close to the skin in the median abdominal line. A small area may be shaved, although this is not necessary. Disinfect with an application of iodine in alcohol.

3. With the left forefinger and thumb pinch up the entire thickness of the abdominal parietes in a triangular fold, and slip the peritoneal surfaces over each other to ascertain that no coils of intestine are included.

4. Grasp the syringe in the right hand and insert the needle into the fold near its base.

5. Release the fold and inject the fluid. If a swelling forms, this shows that the needle is in the subcutaneous tissues, and another attempt should be made to enter the peritoneum.

6. It may be difficult to pinch up the parietes without including the intestine. In such case straighten out the animal and stretch the skin between the left forefinger and thumb. Insert the needle obliquely until it is beneath the skin. A slight thrust suffices to pierce the peritoneum, when the abdominal muscles will be felt to relax. Withdraw the needle slightly and inject the fluid.

7. Seal the wound with a touch of collodion.

CHAPTER IV

THE PRESERVATION OF SERUMS—METHODS

It is well to remember that serum collected shortly after a meal is likely to be cloudy or opalescent; it is therefore advisable that blood be collected several hours after eating or during a period of fasting.

After securing a specimen of blood the container should be set aside and kept at room temperature until the serum separates. If the serum is to be used at once blood may be collected in centrifuge tubes, allowed to coagulate, and then broken up, as gently as possible, with a sterile glass rod and thoroughly centrifuged. On account of the mechanical rupture of erythrocytes such serums are usually tinged with hemoglobin. After serum has separated from the clot it should be transferred to another tube or, if this is not immediately possible, the container should be placed in the refrigerator to retard hemolysis, which may soon occur and render the serum unfit for many purposes.

Small amounts of serum are best removed from the clot with a capillary pipet and teat or with an ordinary graduated pipet with rubber tubing and mouth-piece, in order that one may see exactly what he is doing and not disturb the clot. As a perfectly clear serum is always to be desired, serums mixed with corpuscles should be centrifuged.

It may be stated, as a general rule, that all normal and immune serums should be collected as aseptically as possible and handled in a careful and aseptic manner, so as to insure a clear and sterile product. Notwithstanding the method of preservation all serums should be kept in a refrigerator or ice-chest at a low temperature.

PRESERVATION OF NORMAL SERUMS

Normal serums that are to be used for purposes of immunization are best preserved in small amounts in separate ampules, or in a large stock bottle holding from 100 to 200 c.c. and well stoppered. In the production of precipitin serum, for example, sufficient serum of an animal may be obtained at a single sitting for the whole course of injections, and this serum is best preserved in separate ampules. Each ampule should contain sufficient serum for one injection, and be sealed and marked. In this manner the risk of contaminating a stock bottle is obviated. In the preservation of normal serum or the serum of luetics to be used as controls for the Wassermann reaction, it is better to store them in small amounts in sterile ampules.

As a rule, it is best not to add a preservative to serums that are to be used for purposes of immunization, for, if the dose of serum is large, enough preservative may be injected to place the health of the animal in jeopardy. However, chloroform may be added in proportion of 1 : 10 or 1 : 20, provided the serum is placed in the incubator or heated in the water-bath at 40° C. for fifteen minutes in order to drive off the chloroform previous to injection.

PRESERVATION OF IMMUNE SERUMS

Immune serums may be preserved either in the fluid or in the dry form. **Preservation in Fluid Form with Antiseptics.**—Practically any immune serum may be preserved in the fluid state by adding a suitable preservative

in the proper dose without exerting any deleterious influence on the antibody content. The exceptions to this general rule are the precipitin serums, because these should be crystal clear, and a preservative may render the serum slightly cloudy. According to Uhlenhuth, Weidanz, and Wedemann, such serums should be filtered through a sterile Berkefeld filter and then stored without adding an antiseptic.

Various antiseptics have been advocated for the preservation of serums. Hemolytic serum is well preserved by adding an equal amount of chemically



FIG. 49.—A SMALL BERKEFELD FILTER.

The fluid to be filtered is poured into the glass cylinder surrounding the earthen or porcelain "candle." Negative pressure within the candle is produced by the water pump, which exhausts the air from the flask. The filtrate is collected in the test-tube within the filter flask. All parts are readily sterilized in an Arnold sterilizer or autoclave. The sterile cotton plug prevents air contamination.

pure glycerin to the serum after it has been inactivated by heating at 55°C . for a half-hour in a water-bath. The addition of 0.1 c.c. of a 5 per cent. solution of phenol in salt solution to each cubic centimeter of immune serum usually suffices to keep the fluid free from contamination, and produces only very slight, if any, clouding. Likewise, the addition of 2 per cent. formalin in a 5 per cent. solution of glycerin in normal salt solution, in the proportion of 1 : 10, makes a very useful antiseptic. Neither lysol nor trikresol should be used in the preservation of a serum, as they are more likely to produce clouding than does phenol.

In order to avoid the formation of precipitates when cresol is added to serum, Krumwiede and Banzhaf¹ have recently advocated the use of a mixture of equal parts of ether and cresol. On the addition of such a mixture to serum the solution floats on the surface, causing a slight haze at the point of contact. If shaken immediately no precipitate forms or, at most, very little. The addition of ether to serum for therapeutic purposes does not appear to be harmful and the mixture is recommended for the preservation of antitoxins and other sera.



FIG. 50.—A FILTER.

The cotton plug in the "candle" is removed and the fluid poured within the candle (hollow). The water is then turned on and the stop-cocks are opened; a vacuum is produced within the flasks, which draws the fluid through the candle. The filter is readily cleansed and sterilized (autoclave) and is quite efficient.

Preservation in Fluid Form by Bacteria-free Filtration.—If serums are to be preserved in fluid form without the addition of an antiseptic, special precautions in bleeding, collecting, and separating should be observed. If contamination has probably occurred, the serum should be filtered through a sterile Berkefeld filter (Fig. 49). If the serum proves to be sterile it is transferred, with the aid of a sterile pipet, into ampules of 1 c.c. capacity. These are sealed hermetically and kept in the refrigerator.

Small amounts of serum may be lost in a large filter and a smaller

¹ Jour. Infect. Dis., 1921, 28, 367.

apparatus should therefore be used. The filter shown in the accompanying illustration (Fig. 50) is quite serviceable, as the flask and earthen candle-filter may be wrapped in a towel and sterilized in the autoclave. The apparatus may carefully be attached to a suction pump and the serum pipeted off into the hollow of the candle and filtered, the filtrate being removed at the completion of the process by another sterile pipet.

Method of Cleaning Filter.—Care should be exercised regarding the reaction of Berkefeld filters and particularly new filters. Before use they should be boiled in distilled water at least three times for five minutes each time, and scrubbed with a small soft brush after each boiling. After the filter is set up hot, neutral, distilled water should stand in it for about five minutes and then washed through under gentle pressure until the fluid is clear and neutral to phenolphthalein, when the filter is ready for use. After use the filter should be boiled in distilled water, scrubbed, and dried in the air.

Preservation in Fluid Form by Freezing.—Freezing a serum often renders it cloudy or causes a precipitate to be deposited, and interferes with the usefulness of a serum that should be absolutely clear. Freezing is the only practicable method so far devised for the preservation of thermolabile substances, such as complement. A small apparatus, named the "Frigo," has been devised for this purpose by Morgenroth. A satisfactory apparatus may be made by constructing a wooden box with a smaller sheet-metal-covered inner compartment, the space between them being well packed with sawdust. This inner box is then filled with crushed ice, and the whole is covered with a lid lined with several layers of felt.

Preservation in Powder Form.—When serum is poured out in thin layers and dried, it forms yellowish, amorphous masses, that may be collected and ground into a powder, which keeps well and forms an excellent medium for the preservation of many immune serums, especially those of the agglutinating type. Various toxins, such as tetanus toxin and cobra venom, may also be preserved in this form.

The serum or toxin may be spread out in thin layers on large glass plates, or placed in shallow dishes and dried in the incubator. After a few hours the dried serum, which adheres only slightly to the dish, can be removed with a spatula and placed in a mortar, and ground and stored in sealed tubes.

The drying process is better carried out *in vacuo*, and the large serum institutes are provided with these special drying apparatus. A simple form may be prepared after the method of Taczé as follows: Place a large glass bell-jar with a ground base and a large opening at the top on a polished iron plate. Set this on a large tripod, as this will facilitate heating with a Bunsen burner. The serum is placed within the jar in a shallow dish, and the jar fastened to the iron plate with hot paraffin or wax. The opening at the top is closed with a three-holed rubber stopper: one hole carries a thermometer; a second is connected with a manometer (not absolutely necessary), and the third carries a bent glass tube which is connected, by means of thick-walled rubber tubing, to a suction pump. A low flame is kept burning so as to keep the temperature at about 35° C. The degree of vacuum secured makes little difference, and usually that obtained with an ordinary water-suction pump, allowing for leaks in the tubing, is sufficient, rendering manometric measurements unnecessary.

I have secured equally good results in evaporating serums and tissue extracts with an ordinary electric fan enclosed in a properly sized oblong wooden box to concentrate the air current and exclude dust (Fig. 51).

The dried serum should be dissolved in sterile normal salt solution before it is used.

Preservation in Dried Paper Form.—This is a very serviceable method for preserving hemolysins and, to a lesser extent, agglutinins. In the preservation of hemolytic amboceptor Noguchi advises the use of Schleich and Schull's paper No. 597. The paper is cut into squares about 10 by 10 cm., and saturated with the serum which, after preliminary titration, has been found satisfactory. Sufficient serum is added to wet the sheets evenly, any excess of serum being absorbed with other sheets of paper. Each square is placed separately upon a clean sheet of unbleached muslin and dried at room temperature. When thoroughly dry the squares are care-

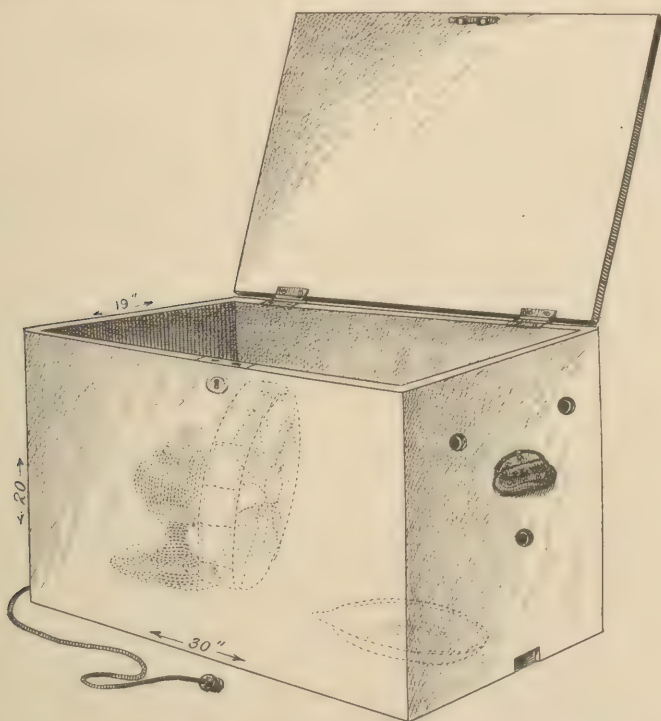


FIG. 51.—A CONVENIENT BOX FOR DRYING SERUMS, EXTRACTS, ETC.

fully ruled off with a hard pencil into widths of about 5 mm. and cut into strips. The paper is then standardized and preserved in dark glass vials in a cool, dark place.

Preservation in the Living Animal.—In the living animal an immune serum may be preserved by removing a small amount of blood from time to time as needed, the titer being preserved or raised by occasional injections. This method, however, may be unsatisfactory and expensive, especially with the smaller animals, as they frequently show a marked tendency to sicken and die, or may succumb to anaphylaxis. After a time, too, they fail to respond to injections with the formation of antibodies, a condition ascribed to atrophy of the cell-receptors (receptoric atrophy).

PART II

CHAPTER V

INFECTION

Parasitic Causes of Disease.—Broadly considered, the causes of all diseases may be grouped in four classes as follows: (1) Vegetable and animal parasites, or the products of their activity; (2) chemical agents of non-parasitic origin, as the inhalation and swallowing of poisonous gases and dust in the industrial diseases; (3) physical agencies, as trauma and heat, and (4) those of unknown etiology.

The subject of infection concerns all diseases caused directly or indirectly by vegetable and animal parasites; directly, as in the infectious diseases, and indirectly, as for example, by the poisons believed to be produced by the bacterial flora of the intestines under certain abnormal conditions.

Infection is not to be confused with mere surface contamination, and many factors are to be considered in relation to both the parasite and the resistance of the host; this chapter deals with the subject of the mechanism of infection by vegetable and animal parasites, and the succeeding chapter with the mechanism of the production of disease by these parasites.

Disease may be caused, therefore, by both vegetable and animal parasites as follows: (1) By the pathogenic bacteria; (2) by the pathogenic higher plants or microfungi; (3) by pathogenic microparasites of animal origin as some of the protozoa, etc.; (4) by some of the larger animal parasites, as the worms, and (5) by unknown filterable viruses of plant or animal origin.

Our knowledge of the mechanism of infection is largely based upon studies with the pathogenic bacteria; much of this information is applicable to the mechanism of infection with microfungi and protozoa and to infestation with the larger animal parasites.

Throughout this chapter I have used the word *microparasite* as including the bacteria, microfungi, animal parasites of microscopic size with particular reference to the pathogenic protozoa, and the filterable viruses; the word *parasite* has been used to include these and likewise animal parasites of macroscopic size.

Invasion and Contamination.—The skin and adjacent mucous membranes contain numerous microparasites, and under normal conditions these may invade the tissues, but they are usually quickly destroyed and unable to proliferate, *so that mere invasion does not necessarily constitute infection.*

Unfortunately, custom has sanctioned the use of the term "infection" as synonymous with contamination. The bacteriologist may speak of the air, water, or his culture-medium as being infected when they contain micro-organisms, or in other words, are not sterile; similarly the surgeon may speak of a knife or splinter of wood as being infected, whereas, while these may be infective or capable of producing infection, it is more accurate to speak of them as being *contaminated*. In the early days of bacteriology, the mere presence of micro-organisms in or on the skin and mucous membranes was regarded as equivalent to infection. It is now well known that

a person may harbor various micro-organisms, such as staphylococci, streptococci, and pneumococci, without apparent injury to the host, and this surface contamination, or even occasional invasion of the tissues, does not necessarily indicate that the host has been, is, or will be ill.

Definitions.—*When, however, microparasites have passed the normal barriers of the skin or mucous membranes and have invaded and proliferated in the deeper tissues, the process is spoken of as an infection.*

By common consent, the term *infestation*, or infestment, is being applied in a similar manner to the presence and growth of animal parasites of macroscopic size, as the intestinal worms; thus, the intestine may be *infected* with *Bacillus typhosus*, and *infested* by *Tænia saginata*.

A microparasite may be intimately associated with and have its normal habitat in a certain part of the body and do no harm until special conditions arise, when it may rapidly invade the tissues and produce infection; this condition has been described by Adami as a *subinfection*, and is illustrated by the constant presence of staphylococci and streptococci in the tonsils of most persons, usually harmless, but capable, under special conditions, of producing severe and even fatal infection.

The abnormal state resulting from the deleterious local and general interaction between a host and an invading parasite, with consequent tissue changes and symptoms, constitutes an infectious disease.

As has previously been stated, not every invasion of the deeper tissues by microparasites results in injury or disease. A certain number of bacteria and protozoa are constantly gaining admission to the deeper tissues of the alimentary and the respiratory tracts without producing apparent injury to the host, as they tend to be destroyed very soon after they gain entrance. Furthermore, bacteriologic studies of lymphatic glands and other tissues removed during life or soon after death at autopsy, not infrequently show the presence of diphtheroid bacilli and other micro-organisms possessing feeble or no demonstrable pathogenic powers and indicating that various bacteria may gain access to the deeper tissues without producing a true infection. The terms *invasion* and *infection* are not, therefore, synonymous. Every true infection is accompanied by local changes, although these may be so slight as to escape notice; an infectious disease is practically made up of similar phenomena, but these are of an exaggerated or marked degree.

The hygienist distinguishes between: (1) *Sporadic*, or isolated, cases of infection; (2) *endemic*, in which a certain microbic disease affects the inhabitants of a given area year after year, and (3) *epidemic*, in which a disease appears suddenly and affects a large number of inhabitants, the number of cases rapidly increasing and decreasing. Among the lower animals equivalent terms for the types just described are *sporadic*, *enzootic*, and *epizootic*. A *pandemic* disease is one that is epidemic over a large territory.

In all infections there are two inseparable factors to be considered:

1. The offensive forces of the infecting agent, dependent upon its pathogenic or disease-producing nature and its power of defending itself against the antagonistic forces of the host and of thriving under these conditions.
2. The resistance offered by the host and mainly dependent upon certain physical or non-specific local factors or specific antibodies, which constitute the defensive mechanism, or immunologic factors.

The former is concerned with the general subject of infection and the latter with that of immunity.

Parasites and host may live together in apparent harmony, owing to the ability of the host to restrain the activity of the parasite and neutralize its injurious effects or to an absence of infectivity on the part of the parasite

until the vital resistance of the host is diminished or the pathogenicity of the parasite is increased, when the neutral relations are disturbed and infection occurs.

RELATION OF INFECTION TO IMMUNITY

From what has been said it is apparent that the subject of *infection forms the basis for the study of immunology*, for, paradoxical as it would at first appear to be, infection must usually have occurred in order that immunity may be acquired. This relation is not always apparent; for instance, man and some of the lower animals may possess a natural immunity to a certain parasite because of the presence of various physical or non-specific defensive factors, or to specific antibodies produced as the result of an earlier and unrecognized infection, or even one that has been inherited; under any circumstances, however, natural immunity is usually relative and seldom absolute. In passive immunity the same conditions are generally operative, and the antibodies present in the serum used to confer a passive immunity are produced in some other animal as the result of an active infection.

It may be stated, therefore, that specific antibodies are produced only by stimulation of the body cells, and that this stimulation is furnished by the infecting agent either in living, disease-producing form, or in a modified and attenuated state, *i. e.*, in the form of a vaccine; thus it will be seen that infection and immunity are intimately associated, and that, generally speaking, there can be no pronounced protection unless infection has taken place.

SOURCES OF INFECTION

Parasites, and particularly microparasites, are to be found everywhere. For general purposes they may be roughly divided into two classes—saprophytes and pathogens. The *saprophytes* are those parasites which thrive best in dead organic matter and perform the very important function of reducing, by their physiologic activities, highly organized material into those simple chemical substances that may again be utilized by the plants in their constructive processes, and in this manner maintain the important chemical relation between the animal and the plant kingdom. *Pathogens*, on the other hand, find the most favorable conditions for growth and activity upon the living tissues of higher forms of animal life. They include most of the pathogenic, or disease-producing, bacteria and animal parasites.

No marked separation between these two divisions can be made, as numerous species occupy a transition point between the two. The terms are merely relative, and parasites ordinarily saprophytic may develop pathogenic powers when the resistance of the host is sufficiently reduced by another infection, fatigue, exposure, or other deleterious influence. In other words, a pathogen or pathogenic microparasite is one that can grow in the living tissues because the immunologic defenses of the host are not sufficiently strong to resist it; in most cases, however, as will be pointed out further on, a higher degree of immunity can be produced artificially, rendering the microparasite in question relatively harmless for that particular animal. Similarly, under certain circumstances, the resistance of the body, or of a part of it, may be broken down to such an extent that micro-organisms ordinarily regarded as saprophytes may gain access to the deeper tissues, flourish, and produce disease.

Accordingly, no fundamental distinction between pathogenic and non-pathogenic parasites can be made. Any apparent differences are due not

only to various degrees of pathogenicity possessed by the parasite, but also to the different degrees of resistance against their attacks, since a microparasite that is highly pathogenic toward one animal may be quite harmless to another.

CONTAGIOUS AND INFECTIOUS DISEASES

Just as all pathogenic parasites do not possess the same habits of growth, so, likewise, they vary in their vitality and in their ability to proliferate under various conditions when removed from the animal body. Some are able to grow only at body temperature or, indeed, only in the human body itself: when removed from these conditions they may retain their vitality for a short period of time, but are unable to proliferate; from this it follows that communication of these parasites and their disease must be direct or immediate, *i. e.*, from person to person, or almost direct, by the conveyance of the infecting agent in the form of *fomites*, such as dust, epidermal scales, or discharges, or as the result of bites of suctorial insects. This form of infection, which requires such direct means of transmission, and of which gonorrhea is an example, constitutes what are known as *contagious diseases*.

Other parasites may be able to preserve their pathogenic powers and proliferate outside of the body at ordinary temperatures, and may even withstand great extremes of heat or cold and various nutritional deficiencies; they may exist thus for weeks, and carry the disease to a second individual through contaminated material. Infections, the result of indirect transmission, are known as *infectious diseases*.

There are no hard-and-fast rules that can be set down in classifying parasitic infections; parasites that are commonly transmitted by one means may, under slightly altered conditions, be transmitted by another. *The usual classification by which certain diseases are classified as contagious and others as infectious should be abolished, and all should be grouped under the term "infectious,"* there being a definite understanding of those cultural characteristics that render infection more likely to occur by direct and immediate contact, and those that may occur in an indirect or roundabout manner. *It may, therefore, be stated that all parasitic diseases are infectious; the term "contagious" may be reserved for those spread or contracted as the result of direct contact.*

EXOGENOUS AND ENDOGENOUS INFECTIONS

Infection may occur as the result of the admission of microparasites to the tissues from sources entirely apart from the individual infected (*exogenous infection*), or from the admission of some of those microparasites living normally and harmlessly on the skin and adjacent mucous membranes, and which, under special conditions, have assumed pathogenic properties (*endogenous infections*).

Exogenous infections are the more usual form, and result from contact with infective material outside the body.

1. Microparasites, such as typhoid and cholera bacilli, and pathogenic amebas, which can live for varying periods of time in *water* and *foods*, are particularly likely to gain entrance through the gastro-intestinal tract. Micro-organisms may be present in milk derived directly from diseased animals or tissues, and when ingested, may produce disease. Thus, for example, the bacillus of tuberculosis may be conveyed in either milk or flesh, young children being particularly exposed to this method of infection.

2. The *atmosphere* may be laden with micro-organisms, which, whether or not capable of proliferating outside of the body, are prone to gain entrance through the respiratory tract, especially through the upper air-passages, the pharynx and tonsils being often the seat of the infection.

3. Micro-organisms capable of existing on the *skin* may gain entrance to the deeper tissues as the result of wounds. Under these conditions of lowered vitality of the local tissues micro-organisms that would otherwise be harmless may become pathogenic and morbidly affect the host, either locally or generally. As the skin is brought so freely in contact with external objects, various microparasites, and particularly the pathogenic cocci, may gain entrance to the dermis. Wounds may be infected by the *teeth and secretions of animals*, or by various *weapons and implements* contaminated with infective material, as, *e. g.*, the virus in the saliva of rabid dogs, or the spores of the tetanus bacillus on rusty nails.

Contact with *unclean objects* of various kinds—eating utensils, catheters, syringes, dental instruments, etc.—may serve to transfer pathogenic parasites from one person to another. This is especially likely to occur if the skin or mucous membrane is abraded, the infecting parasites thus gaining ready access to the deeper tissues. In some infections, however, even this local injury is unnecessary, as the parasite may be able to proliferate and produce lesions on an intact surface, as, for instance, the diphtheria bacillus in the pharynx, and various fungi, such as Achorion, Trichophyton, etc., on the scalp and skin in general.

Microparasites affecting the genital organs are likely to be conveyed directly from one sex to the other in conjugation, or to the child during parturition.

4. *Suctorial insects* may serve as the medium by which microparasites are transmitted from person to person. In most instances the transmission is a purely mechanical process, as witness the transmissions of the plague bacillus in the intestinal contents of the rat flea; in the case of malaria, on the other hand, the interposition of the mosquito is essential to complete the life cycle of the protozoön.

5. Micro-organisms infecting the *placenta* may pass to the fetus by way of the umbilical vein.

Endogenous infections arise as the result of the activity of microparasites having their normal or customary habitat in the body. Such infections do not represent so much an assumption of pathogenic power on the part of the microparasite, as they do a disturbance of the defensive mechanism of the host, whereby the normal relations are disturbed, and microparasites that normally are harmless, become infective and disease-producing. While the disturbance of the defensive mechanism may be general, it is far more likely to be local; an example is that of appendicitis the result of *Bacillus coli* infection following passive congestion due to fecal impaction of the colon.

AVENUES OF INFECTION

Local infection may occur in any portion of the body, and any part may prove the point of entrance of parasites to the body fluids, the result being a general infection. Owing, however, to the peculiar pathogenic properties of different bacteria and their affinity for the cells of certain tissues, coupled with a peculiar tissue susceptibility for certain bacteria or their products, we find that many diseases have regular avenues of infection, and, indeed, in a few instances infection of the human body may be possible only through a particular and definite route. Infections of the

gastro-intestinal, respiratory, and genito-urinary tracts and various sinuses with external openings must be considered as being potentially surface infections. The outer layers do not consist merely of the skin and adjacent mucous membranes, but are made up of all layers covering surfaces and channels, which, however, indirectly communicate with the exterior. In the higher animals there is only one direct channel of communication between the actual interior and the exterior of the body, this being through the fallopian tube of the female, which normally has so fine a lumen and is so well protected that to all intents and purposes it may be regarded as closed. In certain inflammatory conditions of the genital organs, and particularly after parturition, the fallopian tube may be open, and afford a direct route for the transmission of infection from the external parts to the peritoneal cavity.

Living in and on the actual and potential external surfaces are countless micro-organisms, which are for the most part harmless, a few being, however, actually or potentially dangerous.

1. The skin and adjacent mucous membranes, particularly in those portions where warmth and moisture abound, are well adapted to bacterial growth, and their contact with surrounding objects causes a large variety of micro-organisms to adhere to them.

As a result, the bacteriology of the skin is quite complex, since it may lodge micro-organisms from the air, from water, and from soil. A group of cocci and diplococci, particularly the *Staphylococcus epidermidis albus* of Welch, and the various pseudodiphtheria bacilli, are habitually present upon the human skin. When local injury occurs, they may produce minor suppurative lesions, and may be concerned in the production of certain skin diseases, such as eczema, impetigo contagiosa, the pustules of variola, etc.

Other micro-organisms may find temporary lodgment upon the skin, and are in no sense regular inhabitants. For example, the fingers and hands may become contaminated with colon, typhoid, and tubercle bacilli, pneumococci, etc.

The skin forms a very important barrier against the entrance of parasites into the deeper tissues. The greater number of local surgical infections result from the entrance of bacteria into lesions of the skin, although these lesions may be so small as to escape notice.

Certain parasites are capable of producing direct action on the skin without previous existing injury, and especially upon the mucous membranes, where moisture and higher temperature are more favorable to growth. For example, a few of the higher fungi, such as *Microsporon*, *Achorion*, and *Trichophyton*, seem able to establish themselves in the superficial cells and invade the deeper tissues through the hair-follicles; staphylococci may reach the roots of hair-follicles and sweat-glands and set up suppurative conditions; diphtheria bacilli may lodge directly on the intact mucosa of the upper air-passages and cause local necrosis and general intoxication; cholera bacilli may have a similar effect upon the intestinal mucosa; the Koch-Weeks' bacillus and the gonococcus may produce severe inflammation of an intact conjunctiva, etc.

2. The respiratory organs commonly afford admission to certain parasites. The nose may be the seat of local infection with *Bacillus influenzae*, *Micrococcus catarrhalis*, *Bacillus diphtheriae*, and other bacteria; it may be the entrance point for meningococci and the virus of anterior poliomyelitis. Similarly, the entrance of such unknown infectious agents as those of scarlet fever, measles, and smallpox can best be accounted for by assuming that

they were inhaled and later entered the blood; there is much clinical evidence to support the belief that the contagium of scarlet fever is present in the discharges of the upper air-passages of persons suffering from that infection.

Whether or not tuberculosis of the lungs is the result of the inhalation of tubercle bacilli is a much disputed point, but it cannot be denied that this theory most readily accounts for the far greater frequency with which tuberculosis affects the lungs than it does other organs of the body.

Pneumonia, caused by the pneumococcus of Weichselbaum, probably results from the direct inhalation of one of the various types of pneumococci, and bronchopneumonia of children is certainly chiefly inspiratory in origin.

3. The digestive tract may be the portal of entrance of many infections. The mouth usually harbors various fungi and bacteria, which may produce local infections, and either directly or indirectly cause caries of the teeth. The putrefactive changes they may produce is being generally recognized as having an important bearing on the causation and symptomatology of several infections, and a carious tooth has been found the portal of entry of micro-organisms causing a general infection. The tonsils are well known to be the breeding and lodging place of various microparasites causing many general infections, such as acute rheumatic fever, tuberculosis, and possibly typhoid fever. The pharynx may harbor the micro-organisms of diphtheria, pneumococcus angina, etc.

Normally, except for the presence of a few sarcinae, the stomach is practically sterile. Under special conditions, however, typhoid, dysentery, cholera, tubercle, and other infectious bacteria may escape the germicidal effects of the hydrochloric acid, and, reaching the alkaline intestinal contents, which are rich in soluble proteins and carbohydrates, are rendered capable of producing their respective infections.

Although these conditions are primarily of the nature of local infection, there is much experimental evidence to show that bacilli, and particularly tubercle bacilli, may pass through a practically intact intestinal wall and find their way to the lymph-glands or to the blood-stream itself.

Aside from these direct and specific infections, various other micro-organisms, by fermentative action, may alter the intestinal contents and produce toxic products capable of exciting acute and severe toxemias. Some authorities—*e. g.*, Metchnikoff—regard the various types of colon bacilli as producing toxic products responsible for chronic degenerative lesions of the cardiovascular and other organs. The digestive tract is therefore regarded by some pathologists as a constant menace to health, in that it permits bacteria to enter the lymphatic and blood-streams, or to produce toxic substances detrimental to health and longevity. Adami has drawn particular attention to a condition which he terms *subinfection* and which is dependent upon the constant entrance of colon bacilli into the blood, whence they enter the liver, where their final dissolution takes place, appearing as fine, dumbbell-like granules inclosed in the cells.

4. The genital organs are the seat of various local infections that may become wide-spread and general. Normally, the urethra may contain a few cocci which lodge about the meatus; the acid secretions of the vagina are generally inimical to bacterial growth, and the uterus and bladder are usually sterile. But three micro-organisms—the gonococcus, *Treponema pallidum*, and the bacillus of Ducrey—here find favorable conditions for growth, and are usually transmitted from person to person by means of sexual congress. The local gonococcal lesion may be the portal of entry of gonococci into the blood-stream, resulting in wide-spread metastases in

the heart valves and joints. The local syphilitic lesion is quickly followed by general infection. Chancroids alone remain localized, although the initial lesion frequently spreads quite rapidly by continuity of tissues. In rarer instances other micro-organisms, such as the ordinary pyogenic cocci, tubercle bacillus, and diphtheria bacillus, may infect these organs and be transmitted by sexual conjugation.

5. There is considerable controversy of opinion regarding the susceptibility of the placenta and the filtering properties it possesses for various infectious agents. A study of this subject by Neëlow¹ would indicate that the non-pathogenic bacteria do not pass from the mother through the placenta to the fetus. Other pathogenic agents may, however, pass through quite readily; for example, pregnant women suffering from smallpox may be delivered of infants showing active lesions of prenatal infection, and syphilitic infection of the fetus is a well-known condition. Most controversy centers around congenital tuberculosis, and directly opposing views for and against prenatal infections are held by several authorities. Baumgarten is of the opinion that many children are subject to antenatal infection, though the disease infrequently develops in a few of them.

The general subject of antenatal infection and pathology is a field requiring considerable investigation and research.

NORMAL DEFENSES AGAINST INVASION

When the large area of the body that is subject to traumatic injury and accidental infection is considered, it is remarkable that, considering the enormous numbers of various bacteria, infection does not occur more frequently.

Bacterial invasion of the tissues is of frequent occurrence, but in health they do not usually cause infection and tend to be destroyed very soon after they enter the tissues.

It may be well to discuss at this point the factors tending to *prevent invasion*, and leave the consideration of the defensive mechanism whereby the body destroys bacteria after successful invasion and thus prevents infection for the chapter on Natural Immunity.

Of the factors preventing invasion with bacteria and animal parasites, the following are recognized:

1. The structure of the surface layer of epithelium. The epidermal cells offer a mechanical obstacle to invasion. This resistance is naturally more complete where the cells are thickened and most compact. In the depths of glands and in mucous membranes where numerous glands are present, and where the layers are thinner and moisture exists, the barrier is less complete.

2. Surface discharges are potent factors in preventing invasions by: (1) Washing away the microparasites mechanically; (2) by germicidal activity through the presence of various chemical agents, such as acids, which they may contain, and (3) by antiseptic and even bactericidal substances that may be present in the form of antibodies.

The saliva, with its antiseptic and germicidal properties, is potent in preventing infections of the mouth and upper air-passages; when this secretion is diminished, as during the course of high fever, bacterial activity is enhanced, which is evidenced by the development of fetid sordes about the teeth and on the lips.

The acidity of the gastric juice and its germicidal powers are well known

¹ Centralb. f. Bakt., 1902, orig., xxxi, 691.

and appreciated; similarly, the urine, the milk, and to a slight extent, the bile, have been demonstrated by Adami to exert a distinct antiseptic effect upon certain bacteria, such as the *Bacillus coli*.

Surface moisture and discharges about the nose and throat are also potent factors in mechanically removing bacteria from inspired air and no doubt frequently prevent bacterial invasion of the lower respiratory tract, where more mischief may be done.

3. The cells of certain excreting glands may possess bactericidal and excretory powers of value in preventing bacterial invasion (Adami).

MECHANISM OF INVASION

We will now consider the method by which invasion, the first step of what may be an infection, is brought about. In brief, one or all of the normal defenses just described must be overcome; in some instances the parasites, by their inherent disease-producing powers, may accomplish this unaided; in other instances the resistance is overcome by a general lowering of the vitality of the body defenses.

1. Traumatic solution of the surface layers of epithelial cells is a very important factor in the production of infection, as the invading micro-parasites are thus given easier access to the deeper and less resistant tissues. The pathologist or surgeon may, in the course of his work, contaminate his hands with secretions containing virulent micro-organisms, and may yet escape infection unless a small break in the surface epithelium, in the form of a scratch or a needle-prick, is present.

2. As has been previously stated, certain bacteria, notably the diphtheria bacillus, by concentrating at one point, may lower the vitality and cause necrosis of superficial cells of the mucosa lining the upper air-passages, and in this manner induce a local break in the continuity of the epithelial covering. Staphylococci may exert a similar action in the depths of sweat and sebaceous glands, and, indeed, certain fungi, such as the *Trichophyton*, *Microsporon*, and *Achorion*, may attack the intact skin. While, therefore, solution of the surface coverings is a very important source of many infections, it is not essential for the production of all.

3. Alterations of the surface discharges, either in quantity or in quality, may permit bacteria to proliferate freely and produce sufficient toxic matter to affect the surface cells, lower their vitality, and destroy them, with the result that they may gain entrance to the deeper tissues. When the secretions are diminished or altered, as, for example, the saliva during a fever, unless the mouth is carefully and frequently cleansed, it becomes putrescent with bacterial growth. Similarly, catarrhal gastritis, or any other factor tending to lower acidity of the gastric juice, favors infection by this route.

4. Not infrequently bacteria may gain access to the deeper tissues or to an internal organ, and infection may occur without any recognizable solution of continuity of the surface epithelium. In these hidden or "cryptogenic infections" the entrance point of the parasites may be healed over or the infecting micro-organisms may have been carried to the circulating body fluids by the wandering cells.

Not infrequently, in cases of tuberculosis of the cervical and mesenteric glands in children, there may be no signs whatever of local irritation in the fauces or in the intestine to explain the source of infection. The tonsils are now strongly suspected and, indeed, known to be the source of entry of bacteria causing several acute and chronic infections.

The leukocytes, in their phagocytic activities, no doubt play an im-

portant rôle in the production of cryptogenic infections, especially when an excessive number of pathogenic bacteria have congregated at one point, and congestion, increased leukocytic infiltration, and a lowered vitality of the tissues have occurred prior to the invasions of micro-organisms. Wandering cells are commonly found on mucous membranes, gathering up various bacterial and cellular débris. They may carry a virulent micro-organism into the deeper tissues and, although this may not produce an infection, a large number of bacteria so transported may be able to resist destruction and prove capable of causing infection.

5. Aside from the question of local conditions in the process of infection other factors may exert an influence. The temperature of the host may be unsuitable for the growth of a certain parasite, even though it has gained entrance to the deeper tissues; a particular route for the introduction of the infecting agents may be necessary, as in typhoid fever and cholera, which are probably always intestinal infections, and, finally, even after the infecting agent has reached the deeper tissues, extension is prevented by a local inflammatory reaction. In many such instances the question of natural immunity is brought into intimate relation with the subject of infection.

After invasion has occurred some bacteria can best sustain themselves against the defenses of the host at the local point of entry. Such micro-organisms may, however, possess unusual vitality and indirectly, through the lymphatics, find their way to the blood-stream, producing a *bacteremia*. This is a morbid condition characterized by the presence of micro-organisms in the circulating blood.

Some micro-organisms may gain entrance to the general circulation more readily than others, and their mode and route of entry vary in the different infections. It is essential that they possess an unusual degree of invasive power, and be capable of protecting themselves against the manifold defensive factors contained in the blood. Kruse believes that in local infections the high pressure of an inflammatory exudate may force bacteria into the adjacent vessels; that they may sometimes be carried into the deeper tissues, and even into the blood-stream, by leukocytes is not to be denied.

When bacteria have entered the circulation they may act as emboli in the finer capillaries or, being unable to remain in the circulation, may collect in the capillaries of less resistant tissues, proliferating and producing local metastatic lesions, usually purulent in character. The condition thus produced is known as *pyemia*.

Saprophytic bacteria or pathogenic bacteria of feeble invasive powers may be able to grow in diseased tissues, such as gangrenous areas, and may assist in effecting morbid changes, producing toxic products of decomposition which, when absorbed into the body, give rise to a series of toxic phenomena, such as fever, rapid pulse, malaise, etc. This condition is known as *sapremia*, a term that has also been applied to the decomposition of relatively sterile organic material and absorption of the toxic products, as when portions of placenta or fetal membranes are retained in the uterus after childbirth.

The term *toxemia* is employed rather loosely to mean the presence of any toxic material. Its use should be limited to the *condition resulting from the absorption of the poisonous substances produced by the non-invasive bacteria themselves, as in diphtheria and tetanus*. *Septicemia* is the term applied to the presence in the body fluids of toxic products generated by the pyogenic micro-organisms.

MECHANISM OF INFECTION

Since bacterial invasion is of frequent occurrence the question naturally arises, Why are not infections, both local and general, more frequent? Thus, abrasions of the surface epithelium are not uncommon in the presence of active micro-organisms; tubercle bacilli may be inspired, and typhoid bacilli may be swallowed, the altered local conditions affording opportunity for producing infection, and yet the host may escape.

Bacterial invasion, therefore, does not necessarily mean infection, and it may be stated that infection can only take place when—

1. The micro-organisms are sufficiently virulent.
2. When they invade the body by appropriate avenues and reach susceptible tissues.
3. When they are present in sufficient numbers.
4. When the host is generally susceptible to their action.
5. When the micro-organisms are able to resist the defensive forces of the host through special agencies aside from their offensive forces.

Not all these factors must necessarily be present before infection may occur. A micro-organism may be particularly virulent, so that numbers are relatively unimportant; a host or a portion of the host may be so susceptible or vulnerable to infection that a micro-organism of low virulence, which, under normal conditions, would be totally unable to produce infection, may now prove pathogenic.

VIRULENCE

Virulence refers to the disease-producing power of a microparasite, and is dependent upon two variable factors: (1) Toxicity, and (2) aggressiveness, or the invasive power of the microparasites. In most infections usually both factors are operative.

Toxicity is the term applied to the kind and amount of poison or toxin produced. This poison may be readily *soluble*, or *exogenous*, diffusing into the surrounding tissues and being readily absorbable; or it may be *endogenous*, and contained chiefly within the micro-organisms, and be liberated only upon the dissolution of the cell.

Aggressiveness is a term applied to the invasive powers of a micro-organism to enter, live, and multiply in the body-fluids, or, in other words, to the aggressive or progressive forces of the micro-organism in its new environment.

Toxicity is generally confused with aggressiveness, a highly toxic micro-organism being regarded as an aggressive one. For example, the bacillus of tetanus is highly toxic because of the production of a potent soluble poison which gives rise to the symptoms of tetanus, although it is only slightly aggressive, being almost unable to multiply in the tissues. The anthrax bacillus, on the other hand, is highly aggressive, owing to the fact that it usually multiplies to such an extent that it can be found in each drop of blood and in every organ of an infected animal; nevertheless it is but slightly toxic, the animals frequently showing few or no symptoms until shortly before death. The toxicity of a micro-organism should, therefore, be regarded separate from its aggressiveness, although in many infections both factors are so intimately concerned that the term "virulence" may be used to express the degree of pathogenicity or the total disease-producing power.

The virulence of a micro-organism is more or less specific, *i. e.*, the toxin produced by one species is different from that produced by another in the

kind of disease produced and the species of animal infected. Some toxins are active for certain animals only and not for others. Micro-organisms of one group may possess general and common pathogenic properties differing only in degree; those of different morphologic and cultural characters may possess totally different powers.

The virulence of a given species is subject to great variation. A few bacteria almost constantly retain their virulence, even when kept for years under artificial conditions; as an example may be mentioned the diphtheria bacillus; others quickly lose their virulence as soon as they are grown artificially, as, *e. g.*, the influenza bacillus; in others—and probably the larger class—the virulence may be raised or lowered according to the experimental manipulations to which they may be subjected. Variations may also be observed among members of the same group of micro-organisms, and even among individual micro-organisms of the same strain.

Decrease of virulence of a micro-organism may be brought about artificially by repeated growth in or upon culture-media, especially when transfers to fresh media are made at prolonged intervals. This decrease probably depends upon an actual decrease in virulence, and particularly upon the selection, in artificial growth, of the less virulent or vegetative forms which grow actively and soon exceed in number their more pathogenic fellows. Each time the culture is transplanted more of the vegetative and fewer of the pathogenic micro-organisms are carried over, until finally the pathogenic bacteria are entirely eliminated, or their virulence totally destroyed, and the entire culture is composed only of vegetative or harmless forms of bacteria.

Various other agencies lead to artificial lessening of virulence, such as exposure, for short periods of time, to a temperature just under the thermal death-point; exposure to sunlight; exposure to small quantities of anti-septic or germicidal substances; the action of desiccation; subjection to increased atmospheric pressure, etc., these methods being commonly employed in the preparations of vaccines to be used for purposes of active immunization.

The passage of a micro-organism or virus through animals usually increases its virulence, but may modify or attenuate it, as in the case of the passage of smallpox virus through the calf, when it loses forever its power of producing smallpox.

Increase in virulence can best be secured by passing the micro-organism through animals. It is practically impossible, by any means, to make a known non-virulent micro-organism virulent, although it is comparatively easy to increase the virulence of a culture that has become well-nigh non-virulent on account of prolonged artificial cultivation. This fact is worthy of emphasis, and is well illustrated by the large amount of work that has been done in fruitless attempts to render non-virulent, diphtheria-like bacilli virulent by passage through various animals or growth on special culture-media.

In cases where the virulence is slight or absent, experimental manipulations of the culture are directed toward gradual immunization of the micro-organisms to the defensive mechanism of the body of the animal for which the organism is to be made virulent. This is well explained according to the hypothesis of Welch, and will be referred to again in the latter part of this chapter. A number of methods are made use of for this purpose:

(a) *Passage through animals*, which enables the micro-organisms gradually to immunize themselves or adopt certain morphologic and biologic changes enabling them best to resist the defensive forces of the host. Since

these defensive forces vary with different animals, and, indeed, with the various organs of the same animals, it is usual to find that virulence raised by animal passage affects only the animal or the particular organ of a certain animal, and not all animals in general. Thus, in general, the passage of bacteria through rabbits increases their virulence for rabbits and not for mice, dogs, pigeons, etc.; passage through mice may increase their virulence for mice, but not for rabbits, guinea-pigs, etc.

(b) *The use of collodion sacs* for increasing virulence has been advocated, especially by French investigators. When micro-organisms are inclosed in a collodion capsule of the proper thickness and placed within the abdominal cavity of a suitable animal, the slightly modified body juices are able to transfuse through the sac, impeding the development of such micro-organisms as are unable to immunize themselves or withstand the injurious influences. In this manner a race of virulent bacteria are artificially selected which can endure the defensive agencies of those juices with which they have come in contact.

(c) *The addition of animal fluids to the culture-medium* may enable the bacteriologist to maintain or even to increase the virulence of a micro-organism according to the principles of artificial selection. The fluid, either a serum or whole blood, is secured in a sterile manner and added to the medium in a raw or unheated condition. In this manner the micro-organisms are exposed to some of the defensive agencies contained in the juices under these conditions, and this tends to destroy the less resistant bacteria, encourage the more resistant, and at least maintain, for a longer or a shorter time, the virulence of a culture freshly isolated from a lesion or cultivated by animal passage.

THE AVENUE OF INFECTION AND TISSUE SUSCEPTIBILITY

Successful infection of the body by certain parasites can be accomplished only when invasion takes place through appropriate avenues. Thus typhoid, cholera, and dysentery infection seems to take place through the gastrointestinal tract, and doubtfully by inhalation, and not at all through the skin or urogenital system; gonococci usually enter the body through the genital organs or the eye, and not through the respiratory apparatus or through the skin. The route of infection is less important with micro-organisms characterized by great aggressiveness and producing general, rather than local, infections. For example, in most animals anthrax is a general bacteremia, regardless of the route of invasion; plague rapidly becomes a bacteremia, whether the bacilli are inhaled, rubbed into the skin, or reach the lymphatics through superficial abrasions; similarly, local staphylococcus and streptococcus infection may become general, regardless of the route of invasion or the location of the local lesion.

The avenue of invasion is also of importance in determining the form, nature, and virulence of an infection. Thus virulent pneumococci lodging in the pharynx may produce a pseudomembranous angina; in the eye, a severe conjunctivitis; and in the lungs, a pneumonia. When tubercle bacilli gain admission through the skin, they may produce lupus, or a low-grade inflammatory disease rarely terminating fatally. When inhaled, they may produce tuberculosis of the lungs; in the throat they may reach the tonsils and later the local lymphatic glands, etc. When swallowed, they may produce ulceration of the intestines, or pass through the intestinal walls and involve the mesenteric glands, and later the lungs or other organs.

Just as general susceptibility of the host renders infection more likely

to occur, so local susceptibility may be induced by injury and fundamental disorders. These changes may not only furnish pabulum for the invading bacteria, but more especially reduce the local resistance of the body defenses.

Even more important, however, is the predisposition of some pathogenic micro-organisms to attack certain tissues or organs, and the fact that these tissues are particularly weak in defensive power so that the bacteria naturally lodge where conditions are most favorable for their growth.

Selective Tissue Affinity.—While the primary focus of infection is determined largely by the route of invasion, the selective affinity of micro-organisms or their toxins for certain tissues and the inherent tissue susceptibility to the toxins or bacteria are best in evidence in the location of secondary foci or localization of the infection in general bacteremias. Thus, the seat of the principal local lesions in pneumonia is the lungs, and in typhoid fever the lymphoid tissues, especially that of the spleen, and Peyer's patches in the intestine. It is true that mechanical factors may aid in this selection, as, *e. g.*, the occlusion by emboli of micro-organisms caught in the capillaries of organs; but, in general, we must conclude that either (1) micro-organisms tend to be destroyed in every tissue or organ except those that are poor in defensive forces and are susceptible, or (2) that micro-organisms or their products circulate passively through a tissue and do not lodge because they possess no affinity for these cells. In many infections both processes are probably operative, and at least we are led to the very important conclusion, laid down by Adami, that "in infections the body is never involved as a whole. Coincidentally with the growth of the specific germs in individual organs there tends to be a reaction to, and destruction of, the same in other parts."

Nickols and Hough¹ and Reasoner² have isolated strains of *Treponema pallidum* from the nervous tissues that appeared to have selective affinities for the cornea, choroid, and retina of the eyes of rabbits; Noguchi³ has noticed that various types produced lesions in rabbits of certain distinct characters and with considerable constancy. Further and similar studies may show that various strains of *Treponema pallidum* may possess selective tissue affinities and thereby explain the early development of lesions in the central nervous, cardiovascular, and cutaneous systems of different persons. In fact, Levaditi and Marie⁴ has recently reported experiments showing the existence of two distinct strains of *Treponema pallidum*, one producing a large primary and well-defined cutaneous lesion, designated as the dermatropic strain, and a second producing a small and ill-defined primary and secondary lesion, but possessing marked affinity for the tissues of the central nervous system, designated as the neurotropic strain. According to the investigations of Rosenow⁵ various bacteria, and particularly streptococci, exhibit extreme degrees of tissue affinity and produce various constant and distinct lesions in rabbits after inoculation by various routes. This subject is further discussed in the section devoted to Focal Infection.

The numeric relationship of parasites to infection is very important, and the number alone may determine whether or not it shall occur. Usually the normal defensive factors of the body are sufficient to overwhelm one or a few bacteria unless they are especially virulent. When an intercurent

¹ Jour. Amer. Med. Assoc., 1913, 60, 108.

² Jour. Amer. Med. Assoc., 1916, 66, 1917; *ibid.*, 1916, 67, 1799.

³ Jour. Lab. and Clin. Med., 1917, 2, 472.

⁴ Bull. de l'Inst. Pasteur, Nov., 1919, 741.

⁵ Jour. Infect. Dis., 1915, 16, 240; *ibid.*, 1915, 16, 367; *ibid.*, 1915, 17, 219; *ibid.*, 1915, 17, 403; *ibid.*, 1916, 16, 501; *ibid.*, 1916, 18, 383; *ibid.*, 1916, 19, 333; Jour. Amer. Med. Assoc., 1914, 63, 1835; *ibid.*, 1915, 64, 1968.

or chronic disease, malnutrition, or injury renders the host more susceptible than normal, fewer bacteria than would otherwise be required may successfully infect the body. Also with those microparasites with well-marked aggressiveness, such as the anthrax bacillus, a few may be sufficient, if they reach the circulating fluids, to produce infection. Thus, Webb, Williams, and Barbor¹ have found that one anthrax bacillus was sufficient to infect a white mouse, and as few as 20 tubercle bacilli were sufficient in one instance to infect a guinea-pig.

Likewise Kolmer, Schamberg, and Raiziss² have found in experimental trypanosomiasis that the infection of white rats by intraperitoneal injection with varying numbers of trypanosomes, counted after the method of Kolmer,³ greatly modifies the time of appearance of trypanosomes in the peripheral blood and the duration of life, and has an important bearing upon studies in the chemotherapy of experimental trypanosomiasis.

Park has directed attention to the fact that when bacteria are transplanted from culture to culture, under supposedly favorable conditions, many of them die; it is highly probable that when they are transplanted to an environment that is likely to be unfavorable, as are the body tissues with various defensive mechanisms, many more must die. This is an important point to bear in mind in attempting to correlate experimental results with the natural cause of an infectious disease. In the laboratory we reproduce disease experimentally by the immediate injection of millions of bacteria, whereas in nature there is rarely any such immediate overwhelming of the tissues. For example, pneumonia may be produced experimentally in dogs by the injection of a large number of virulent pneumococci directly and at once into the bronchi, yielding a positive result with a micro-organism which, under natural conditions and in smaller numbers, would be relatively innocuous for the animal under observation.

d'Herelle's Intestinal Bacteriophage in Relation to Infection.—According to d'Herelle⁴ there may be an additional defensive agency which microparasites must overcome before infection can occur, and particularly infections of the gastro-intestinal tract. He has found in filtrates of broth cultures of the feces of some normal human beings and lower animals an agent capable of attacking and dissolving living bacteria and particularly dysentery bacilli, which is regarded as a normal agency of defense. In the course of bacillary dysentery, typhoid and paratyphoid fevers, cholera, and other bacterial infections of man and the lower animals this bacteriolytic agent has been found greatly increased, and is regarded by d'Herelle and others as playing an important rôle in recovery from disease.

This substance is regarded by d'Herelle as a living organism of ultra-microscopic size which lives only at the expense of living bacteria, entering them and secreting a diastatic ferment which kills the bacterium and brings about its dissolution by a digestive process; for it he has proposed the name *bacteriophagum intestinale*. Others regard this "bacteriophage" as an enzyme produced by bacteria themselves rather than a living ultramicroscopic parasite. The varied theories concerning its nature and a more complete discussion of the subject is given in the chapter on Ferments and Antiferments. Certainly the presence of an agent of this kind in filtrates of cultures of the feces of some normal healthy human beings and the lower

¹ Transactions Sixth International Congress on Tuberculosis, 1908, p. 194.

² Jour. Infect. Dis., 1917, 20, 10; *ibid.*, 1917, 20, 35.

³ Jour. Infect. Dis., 1915, 16, 311; *ibid.*, 1915, 17, 79.

⁴ Le bacteriophage—son rôle dans l'immunité. Masson et Cie, Paris, 1921. This book has recently been translated into English by Smith and published by Williams & Wilkins Company, Baltimore, Md.

animals, and especially in human beings with bacillary dysentery, may be regarded as proved, although the nature of the bacteriolytic agent is not definitely known. d'Herelle believes that there is but one species of "bacteriophage," but an infinite number of strains, each possessing the power of attacking a certain number of bacteria. While a normal inhabitant of the intestine of all living beings, d'Herelle believes that it may be taken into the circulation and exert its protective and curative action at any point in the body.

It is also claimed by d'Herelle that the virulence of the bacteriophage for bacteria may be increased; certainly it may be enhanced quantitatively as is true of enzymes in general under proper conditions for production. Bacteria, on the other hand, are regarded as capable of acquiring a resistance and protecting themselves against this agent, accompanied by changes in morphology. The "bacilli take a coccoid aspect and become surrounded by a capsule. They become inagglutinable. They resist phagocytosis. They are endowed with a very great vitality and a very high virulence. Loss in resistance is accompanied by a return to normal form and properties."

In relation to infection it may be stated that the work of d'Herelle has shown that in the test-tube at least filtrates of cultures of the feces may digest certain bacteria and that filtrates of these digested bacteria in turn will digest cultures of various bacteria in succession. The lytic agent is filterable; whether it is a living parasite or an enzyme is not definitely known. Whether it exerts an appreciable or important rôle in natural resistance to bacterial infection in general and intestinal infections in particular cannot be stated. Probably it exerts some protective activity and when present must be overcome in cases of infection occurring by way of the gastrointestinal tract; it may also exert a more important rôle in recovery from intestinal infections and particularly dysentery, although, according to Davison,¹ the oral and subcutaneous administration of the filtrates have not proved curative in the treatment of bacillary dysentery of children.

GENERAL SUSCEPTIBILITY IN RELATION TO INFECTION

Under normal conditions the body cells of a host will invariably offer some resistance to invasion and infection by pathogenic microparasites. When, however, any condition that depresses or diminishes general physiologic activity and vitality exists, the host may be unable to master these defensive forces, and accordingly becomes *predisposed or more susceptible to infection*.

Predisposition may be inherited or acquired.

Inherited predisposition may be: (a) **Specific**, or **species susceptibility**, as, e. g., dogs to distemper; cattle to contagious pleuropneumonia; hogs to hog cholera; man to gonorrhea; chancroids, acute exanthemata, typhoid fever, etc. (b) **Racial**, as Eskimos to measles and syphilis, ordinary sheep to anthrax, whereas Algerian sheep are immune, etc. Racial susceptibility is frequently but a lack of acquired immunity; for instance, measles, syphilis, gonorrhea, and other diseases brought by settlers to foreign peoples among whom these diseases were previously unknown, find them peculiarly susceptible and the diseases unusually virulent. (c) **Familial**, i. e., members of a family may, through generations, be unusually susceptible to scarlet fever, tuberculosis, rheumatism, rheumatoid arthritis, metabolic disturbances, etc. (d) **Individual predisposition**, which depends principally upon sex, age, and peculiar tissue susceptibility. Thus, infants are especially

¹ Amer. Jour. Dis. Child., 1922, 23, 531.

prone to contract certain infections on account of the immature development of the body cells, and this susceptibility to infection is further influenced by acquired factors, chiefly malnutrition. On the other hand, very young children enjoy an immunity to several infections, such as typhoid fever, scarlet fever, and even diphtheria, probably due, as Abbott has suggested, to the fact that pathogenic substances that may set up molecular and destructive disturbances in the poorly developed cell have but little effect upon the more inert protoplasm of the immature cell, and that if certain bacteria gain admission to the tissues the cells may destroy them, their toxins not combining with the molecular side-chains, and, as a consequence, not injuring or interfering with the cell functions.

Acquired susceptibility bears a more important relation to infection, and may be due to various factors, most of which lead to a state of reduced vitality, normal physiologic processes being impaired to a greater or less degree.

(a) *Overwork* or overstrain leads to general or local predisposition to disease. Those engaged in hard labor, mental or physical, which involves late hours and inadequate periods of rest and recreation, frequently associated with inadequate nutrition and foul air, are likely to succumb to tuberculosis, typhoid fever, pneumonia, etc.

The influence of overstrain on acute infections has been shown experimentally by Charrin and Roger,¹ who found that white rats naturally immune to anthrax became quite susceptible after being compelled to turn a revolving wheel until exhausted before they were inoculated; similarly of 4 guinea-pigs who were placed in a cage so constructed that they were forced to keep moving for one or two days 3 died in from two to nine days after the experiment. Smears and cultures made from the livers, spleens, and blood gave positive results.

(b) *Previous infection* with the same or another infectious disease may predispose the individual to renewed infection. Thus, some infections, such as erysipelas, furunculosis, acute rheumatism, pneumonia, and influenza, not only fail to leave the body-cells immune, but actually predispose to second attacks. Whether the micro-organisms of these diseases are not all destroyed, but are retained in the system and become active when the general vitality is lowered, or whether a new infection occurs, is not definitely known, and probably either may occur.

One attack of an infectious disease may weaken the tissues and render them susceptible to an infection of a different nature. Thus, the acute exanthemata may follow one another, and tuberculosis may supervene upon any of them.

(c) *Malnutrition* exerts some effect on the resistance to infection. Thus, the terrible epidemics of plague, cholera, typhus fever, and typhoid fever which have followed in the wake of famines in Europe and Asia during the past centuries are examples of the influence of malnutrition as a factor in predisposing to disease. The tendency of marasmatic infants to develop enterocolitis, thrush, bronchopneumonia, and other infections, and of scorbutics to local infections of the mouth, illustrates the influence of insufficient food in decreasing the resistance to disease. Here may also be included local malnutrition, such as loss of nerve or blood supply, predisposing to local infection, especially with pyogenic micro-organisms.

(d) *Diet* produces some variation in the resisting powers to infection. For example, the ordinary wild rat is said not to be susceptible to anthrax unless it is fed for a week or more on coarse dry food, when it becomes

¹ Compt. rend. Soc. de Biol. de Paris, January 24, 1890.

susceptible. Here, of course, malnutrition may come in intimate relationship with diet, as an inefficient diet may greatly lower the general resistance. The influence of diet is particularly noticeable from the fact that the diseases of carnivorous animals are not the same as those that affect herbivorous animals, and that each class is frequently immune to some of the diseases that attack the other.

(e) *Intoxications* of various kinds predispose to infections. Thus, it is a common clinical observation that excessive indulgence in alcoholic beverages predisposes to infections, notably pneumonia. Abbott¹ has demonstrated experimentally that the daily administration to rabbits, of 5 to 10 c.c. of alcohol introduced into the stomach by a tube, renders these animals more susceptible to infection with *Streptococcus pyogenes* and *Bacillus coli*. Wagner, Leo, and Platania have also found animals that under the influence of chloral, phloridzin, alcohol, and curare are more susceptible to infection.

(f) *Exposure* to cold and wet frequently lowers the resistance of man and other warm-blooded animals to infection. The influence of these factors, well illustrated in the etiology of "colds" and pneumonia, is not without experimental foundation. Thus Pasteur found that fowls, which are naturally immune to anthrax, are readily infected if they are inoculated after their body temperature has been reduced by a cold bath. Conversely, Gibier² has shown that frogs, which are also naturally immune to anthrax, are readily infected if their temperature is previously elevated and maintained at 37° C.

(g) *Trauma and morbid conditions in general* may predispose to infection. Thus injuries reduce the local resistance and facilitate local infections that vary with the severity and extent of the trauma. The increased susceptibility of injured joints and pneumonic lungs to tuberculosis, the frequent and oftentimes extensive streptococcus infection accompanying scarlet fever and smallpox, the increased susceptibility of diabetics to furunculosis and local gangrenous lesions of the skin—all show the increased susceptibility of individuals already injured or diseased to infection.

THE DEFENSIVE MECHANISM OF THE MICRO-ORGANISM IN RELATION TO INFECTION

After invasion has occurred, the question of whether or not the microorganism can overcome the defensive forces of the host and prove pathogenic may depend to some extent upon the peculiar defensive factors of the invading microparasite against the offensive mechanism of the host, aside from their toxins or other distinctly offensive forces.

Morphologic and Physiologic Changes of the Micro-organisms.—For example, capsule formation or thickening of the ectoplasm of certain bacteria is evidence of their increased powers of resistance against the opposing forces of the host. The capsule may be quickly lost when the microorganism is cultivated on artificial media, and its virulence be correspondingly lowered, but by repeated animal inoculations a race of capsulated organisms with increased virulence is produced, explaining in a way the mechanism of animal passage in raising the virulence of a given organism. This, however, is not invariable, and indeed, may act in a contrary manner, as the passage of smallpox virus through heifers attenuates and modifies instead of increasing its virulence.

Aggressins.—The micro-organism may actively secrete a material that

¹ Jour. Exper. Med., 1896, 1, 447.

² Compt. rend. Acad. de Sci. de Paris, 1882, xcix, 1605.

overwhelms the defensive forces of the host. This phase of the subject has been studied exclusively by Bail, who sought to prove that the question of pathogenicity of a micro-organism is dependent upon its ability to secrete substances that are able to paralyze the protective forces of the host, especially the leukocytes. These substances are called "aggressins," and they were distinguished by the fact that they were formed by living bacteria and only in the living body. In support of this theory Bail was able to show that substances are present in the exudates of fatal infections, which, when injected in small quantities into another animal with sublethal doses of the micro-organism, would cause a rapidly fatal infection. Later Wassermann and Citron showed that "artificial aggressins" could be prepared by autolyzing bacteria in water or serum. While the subject of aggressins is still unsettled, there is strong evidence to show that they may be the endotoxins liberated by the breaking up of the micro-organism.

The well-known statement of Metchnikoff, that a particular virulent micro-organism is not so readily taken up by leukocytes as is an avirulent strain, may be explained by the fact that the micro-organism, in its virulent state, secretes substances that repel the phagocytes, neutralize the opsonins, or form actual leukocytic toxins. This action may be due to liberated endotoxins or, as Bail claims, to specific secretory substances of the bacterium—the aggressins—specifically formed and liberated by the micro-organism for protection against the host. It is probable that the lysin produced by the "bacteriophage" of d'Herelle is a substance of this kind. As previously stated, d'Herelle regards his bacteriophage as an organism of ultramicroscopic size entering bacteria and secreting a diastatic ferment capable of preventing phagocytosis and producing bacteriolysis. The subject is discussed at greater length in the chapter on Ferments and Antiferments.

Hypothesis of Welch.—Not entirely foreign to this subject is the very interesting hypothesis of Welch. A bacterium may not only produce substances directly inimical to the defensive forces of the host, but it may actually immunize itself against these defensive powers. "Looked at from the point of view of the bacterium, as well as from that of the animal host, according to the hypothesis advanced, the struggle between the bacteria and the body-cells in infections may be concerned as an immunizing contest in which each participant is stimulated by its opponent to the production of cytotoxins hostile to each other, and thereby endeavors to make itself immune against its antagonist."

It is well known that, when freshly isolated from a patient having typhoid fever, the typhoid bacillus resists agglutination, whereas it becomes easily agglutinable after a period of artificial cultivation. It may be assumed that, when active, the bacillus as an infecting agent gradually became more resistant against the agglutinating properties of the patient's serum, and that, when grown on artificial media, it loses this resistance by being removed from the stimulating influence of the infected body.

This hypothesis, however, would go a step further in assuming the possibility of the receptors of the invading bacteria anchoring certain constituents of our body-fluids, and being stimulated to the production of various cytotoxins, which attack the leukocytes, erythrocytes, nerve-cells, liver, kidney, etc. In other words, each bacterium may be conceived as being composed of a central atom group with numerous side chains, just as Ehrlich conceived the hypothetic structure of body cells, and that these side chains, primarily present for the purpose of anchoring food material, may likewise anchor various pathogenic animal substances, with the pro-

duction of substances acting as antibodies to the opposing forces of the host. Welch assumed that these bodies were of the nature of amboceptors, which may become complemented by bacterial complement or by endo-complements of the tissue-cells; this is of secondary importance, and there is no reason why they may not be of different structure, and similar to all three orders of antibodies produced by body cells according to Ehrlich's side-chain theory of immunity. Of further interest in this connection are the investigations of d'Herelle referred to above, who described morphologic changes in bacteria offering resistance to destruction by his "intestinal bacteriophage," an organism of ultramicroscopic size or an enzyme produced by bacteria and preying upon pathogenic and non-pathogenic bacteria themselves. These investigations have emphasized anew the fact that bacteria possess intricate mechanisms of defense against destruction by various chemical and physical agents. As stated by d'Herelle, "although the bacteriophage is capable of acquiring a virulence for the bacterium, the bacterium on its side is capable of acquiring a resistance to the bacteriophage. The virulence of the one and the resistance of the other are not fixed, but are essentially variables, being enhanced or attenuated according to the inherited properties of each of the two germs, and according to the circumstances of the moment which favor the one or the other of the two antagonists."

This hypothesis may also possibly explain certain instances of so-called *species* and *organ virulence*, whereby the virulence of an organism artificially increased by repeated passage through animals of the same species, does not manifest this increased virulence for animals of different species. If, for example, the virulence of the chicken cholera bacillus is increased by repeated passage through the chicken, the increase of virulence affects this animal, but does not affect the guinea-pig. Certain organs may likewise be subject to a similar selective virulence if the increase in virulence has been induced by the specific intervention of those organs and this selective virulence shows itself, irrespective of the manner in which the infection was produced.

That virulence of this order is playing an important rôle in the processes of infection is a theory supported by the discovery that different strains of the same species of bacteria are found to produce characteristic lesions, and while this affinity for a certain organ may be natural and inherent, there can be no doubt that it may also be experimentally induced and acquired. For example, according to Rosenow, a certain strain of streptococcus will produce arthritis; another, endocarditis; another, gastric ulcer, etc.

This remarkable species and organ specificity may be due to the fact that the bacteria of a particular culture have been immunized against defensive forces of a particular animal host or a certain organ of the host, so that, when introduced, they thrive as a result of their special and acquired offensive forces. On the other hand, the specificity may be due to the fact that the bacteria have been accustomed to a certain nutriment furnished by a particular species or organ, and that they cannot thrive unless they receive this special nutriment, and, as a result, the species or organ fulfilling this requirement will become the special seat of infection (Simon).

MIXED INFECTION

Several different micro-organisms may produce infection at the same time, or one may follow the other or others and produce secondary infec-

tion. A disease, as amebic dysentery, may be due to the combined activity of an animal parasite and a bacterium or several varieties of bacteria. The combined effects, upon the tissues of the host, of the products and action of two or more varieties of pathogenic bacteria, and also of the influence of these different forms on each other, are of great importance in the production of disease. The metabolic products of one bacteria may neutralize or accelerate the action of an associated species, or combine to form a new substance entirely different from its antecedents.

Thus, pyogenic cocci affect anthrax bacilli in an injurious manner; on the other hand, aërobic bacteria accelerate or make possible the growth of anaërobes by absorbing uncombined oxygen. Tetanus bacilli will not grow outside of the body in the presence of oxygen unless aërobic bacteria are associated with them; not infrequently tetanus bacilli and their spores would not develop in wounds were it not for the presence of the aërobic bacteria introduced with them; this factor is of much importance, especially in tetanus produced by cowpox vaccine, where, through careless treatment of the lesion, both tetanus bacilli and pyogenic cocci are admitted to the wound.

Again, it may be found that one micro-organism increases the virulence of another; thus, the scarlet-fever virus is favorable to the development of streptococci.

Generally all infections of mucous membranes are mixed infections. Numerous bacteria are present upon the mucosa of the air-passages and gastro-intestinal tract; these are usually harmless unless the resistance of the host is lowered in some manner, in which case not only one but several varieties of these bacteria invade the tissues and cause infection. When one pathogenic micro-organism, such as the typhoid bacillus, has caused the primary infection, because of the local and general conditions of lowered vitality of the tissues, these otherwise saprophytic bacilli tend to intensify the infection. Blood infections, on the other hand, are usually due to one form of bacteria, and even when two or more varieties are introduced, only one, as a rule, is capable of surviving and developing. The products of certain bacteria, on the other hand, may immunize the host against infection with other bacteria, for, as shown by Pasteur, attenuated chicken-cholera cultures may produce immunity against anthrax. In the intestine harmless varieties of bacteria may be made to crowd out more dangerous ones; this is exemplified by the ingestion of soured milk which contains lactic acid bacteria, as advocated by Metchnikoff.

FOCAL INFECTION

In a general manner bacterial infections may be divided into two main groups. In one group the effects of infection are soon manifest by symptoms of disease and this group includes the acute infectious diseases and a host of local infections as those following trauma, surgical manipulations, or occurring without demonstrable predisposing factors other than exposure.

Focal Infection.—In a second group micro-organisms gain access to the tissues at a certain place and produce a localized and confined infection without symptoms, or such slight symptoms as are commonly disregarded; from these *primary foci* or localized areas of infection, however, the micro-organisms or their products may gain access to the lymph or blood streams and produce more serious *secondary foci* or metastatic infections in neighboring tissues or distant and unrelated organs. This is called *focal infec-*

tion, the primary focus being defined by Billings¹ as "circumscribed area of tissue infected with pathogenic micro-organisms," and the metastatic lesions as secondary foci.

The subject has deservedly attracted considerable attention in recent years in both the medical and dental professions; unquestionably focal infection is responsible for many infections and diseased states of hitherto indefinite or unknown etiology. The detection of the primary focus or foci and treatment of primary and secondary foci, are subjects of considerable importance and worthy of closer clinical and laboratory investigation.

Primary Foci.—Billings states that "primary foci of infection may be located anywhere in the body. Infection of the teeth and jaws, with the especial development of pyorrhea dentalis and alveolar abscess, infection of the faucial and nasopharyngeal tonsils and of the mastoid, the maxillary and other accessory sinuses are the most common forms of focal infection. Submucous and subcutaneous abscesses including the finger and toe nails are occasional foci. Chronic infection of the gastro-intestinal ulcers and intestinal stasis due to morbid anatomic conditions; chronic infection of the genito-urinary tract, including metritis, salpingitis, vesiculitis, seminalis, prostatitis, cystitis, and pyelitis, are not uncommon forms. Infected lymph-nodes, which are secondary to the primary foci named, become additional depots of local infection. The secondary lymph-node infection may persist after the etiologic, distal, primary focus has been removed or has spontaneously disappeared. Other secondary foci may appear in various tissues as a part of the general or local disease which results from a primary focus. The tissues so infected may constitute new foci, which in part explains the chronicity of many local and general infections." *Primary foci are especially apt to develop along the upper air-passages, bacteria from the mouth and nose gaining access to crypts in the tonsils and adenoid tissue, to the apices of teeth, through necrotic dentin and pulp canals, and to the nasal accessory sinuses.* In general terms the tonsils and adenoid tissue should first be suspected in searching for primary foci in children and adolescents; the gums, apices of teeth, and bronchi in adults and particularly those over forty years of age. Of course, not all individuals with abscesses at the roots of one or more teeth detected by roentgenologic ray study or with severe gingivitis (oral sepsis) show the effects of focal infection; there are many factors governing focal infection which are not as yet understood and, unquestionably, natural immunity and virulence of the micro-organisms are important factors influencing the development of secondary foci.

Etiology.—As is to be expected a variety of micro-organisms may be responsible for the primary foci, including streptococci, pneumococci, staphylococci, and micrococcus catarrhalis. Of all bacteria so far identified, however, streptococci are certainly the most important, by reason of the researches of Rosenow and others who have found streptococci in primary and secondary foci more frequently than other micro-organisms. Of considerable importance in this connection is the possibility of streptococci growing in primary foci acquiring specific pathogenicity for certain tissues in the nature of tissue tropism or elective tissue affinity. Rosenow,² using special methods, has isolated streptococci from the lesions in acute rheumatic arthritis, cholecystitis, appendicitis, gastric and duodenal ulcer, herpes zoster, and erythema nodosum, which were low in virulence and similar to each other in morphologic and cultural characteristics, but when

¹ The Lane Medical Lectures on Focal Infection, D. Appleton & Co., 1916.

² Jour. Amer. Med. Assoc., 1914, 63, 903; *ibid.*, 1835; *ibid.*, 1915, 65, 1687.

injected into animals, each strain tended to localize electively in the tissue from which it was isolated. In most instances some joint involvement also occurred in the experimental infections, indicating that the vascular anatomy of the joints is an important factor in the production of infective arthritis. It is probable that the micro-organisms in the foci may be affected by changing biochemical properties of the tissues and become accustomed to a special chemical environment which, in addition to trauma and virulence, determines the tendency of a micro-organism to show elective tissue tropism. This subject of selective tissue affinity has been previously discussed on page 71; Rosenow's observations require more general confirmation, but are of great interest in connection with this very important subject of focal infection. The investigations of Davis¹ and Means² tend to show that evidences of apparent selective tissue affinity are to be explained rather on the basis of anatomic structure, susceptibility, and trauma of the tissues.

As previously stated, bacteria or their toxins from a primary focus, are believed to produce secondary foci of infection by direct extension or by distribution by the lymphatic and blood streams. Cultural studies indicate that the bacterium rather than its toxins, are actually transported and responsible for the secondary foci.

Secondary Foci.—Many different tissues and organs may become the seat of secondary foci; probably the joints are involved more frequently than any other tissue due, in part to the vascular anatomy of these tissues favoring the occurrence of bacterial embolism. Iritis, and particularly rheumatic iritis, may possibly be due to focal infection; Rosenow³ and Irons, Brown and Nadler⁴ have successfully produced iritis experimentally in rabbits by injecting streptococci intravenously, and it is probable that many cases of iritis regarded as idiopathic are due to bacterial embolism from some primary focus of infection. Certain affections of the nervous system have been ascribed to focal infection by Rosenow⁵ and Hall.⁶ Judson Daland⁷ and Lewellys Barker⁸ believe that any of the following may be caused by oral sepsis and focal infection: infectious arthritis, hypertrophic osteo-arthritis, local osteomyelitis, myositis, acute infections, endocarditis, secondary anemias, multiple neuritis, and various lesions in the gastro-intestinal, urogenital and endocrinic systems. Ravitch⁹ believes that certain dermatoses may be ascribed to focal infection; Grulee and Gaarde¹⁰ have described cases of acute hemorrhagic nephritis occurring in children probably secondary to a preceding acute tonsillitis. These references serve to show the importance of focal infection in relation to the etiology of a wide variety of diseases demanding most careful study and especially from the standpoints of diagnosis and treatment.

INFECTION WITH FUNGI

A large number of parasitic fungi are known to produce disease in man and the lower animals, the subject of mycology being almost as extensive

¹ Jour. Amer. Med. Assoc., 1912, 58, 1283.

² Archiv. Int. Med., 1918, 22, 617.

³ Jour. Infect. Dis., 1915, 17, 403.

⁴ Jour. Infect. Dis., 1916, 18, 315.

⁵ Jour. Amer. Med. Assoc., 1916, 67, 662.

⁶ Jour. Amer. Med. Assoc., 1917, 69, 689.

⁷ Dental Cosmos, May, 1916; Canada Lancet, August, 1917

⁸ Jour. Dental Research, 1920, 2, 43.

⁹ Jour. Amer. Med. Assoc., 1916, 67, 430.

¹⁰ Jour. Amer. Med. Assoc., 1915, 65, 312.

as that of bacteriology, although less important, because so few of these infections are of a dangerous and fatal character. According to Castellani and Chambers¹ the parasitic fungi for man are practically all found among the Phycomycetes, the Ascomycetes, and the Fungi imperfecti.

The majority of these pathogenic microfungi produce infections of the skin and adjoining mucous membranes, as, for example, ring-worms of the hairy and non-hairy portions of the skin (*Microsporon audouini*; *Trichophyton tonsurans*, etc.), favus (*Achorion schoenleini*), *Tinea versicolor* (*Microsporon furfur*), thrush (*Monilia* or *Oidium albicans*), sporotrichosis (*Sporotrichum beuermanni*), and many tropical diseases. Others may infect internal organs, as well as *Aspergillus fumigatus* (aspergillosis of the lungs, eye, ear, nose, wounds, and skin), *Nocardia bovis*, and other varieties (actinomycosis of nasal mucosa and lungs and actinomycotic mycetomas), and other fungi producing disease primarily or gaining access to the tissues as secondary invaders.

Practically all that has been presented in this chapter on the mechanism of infection with bacteria is applicable to infection by the pathogenic fungi. Curiously, investigators in the field of mycology have devoted their efforts almost exclusively to morphologic and cultural characteristics of the various fungi without giving much attention to the mechanism of infection.

They are usually transmitted by direct contact from man to man, man to lower animal, or, more commonly, by a lower animal to man. Infection is apparently governed by the same principles as is infections by bacteria, among which virulence, avenue of infection and tissue susceptibility, intimacy of contact as influencing numeric infection, invasiveness and natural immunity of the skin in different parts of the body, among races of people, and even between the child and adult of the same race, are important factors.

In this connection mention may also be made of the *phytotoxins*, or toxic substances, derived from certain plants closely resembling the bacterial toxins and possessing lytic and agglutinative properties for the corpuscles of many animals. These will be discussed in more detail in the following chapter.

INFECTION WITH ANIMAL PARASITES

As previously stated many animal parasites of microscopic and macroscopic size are known to infect or infest man and the lower animals. A great number of saprophytic and pathogenic animal parasites have been studied and classified, although the mechanism of infection by those of microscopic size has not received much attention, most effort being devoted to a study of the morphology, life history, and host or hosts of the respective organisms.

Among the pathogenic animal parasites the Protozoa and worms are the most important, particularly the former. Various spirochetes and treponemata (syphilis, yaws, relapsing fever, infectious jaundice, and rat-bite fever), Leishman bodies (kala-azar, oriental sore, etc.), trypanosomes (sleeping sickness, Chagas' disease), amebæ (dysentery, Craigiasis), malaria, intestinal flagellates and ciliates, and other sporozoa, flukes, tapeworms, hookworms, filariæ, and trichina worms are of importance in connection with the subject of infection and investation with animal parasites.

Infection with animal parasites is similar in many respects to infection with bacteria. Owing to the difficulty of isolating and cultivating these parasites *in vitro*, our knowledge of their toxic properties is somewhat

¹ Manual of Tropical Medicine, 3d ed., 1920, William Wood & Co.

meager. Most attention has been given to a study of their life history and the modes of transmission.

Primary infection with animal parasites is often facilitated by, or in some instances only rendered possible through, the intervention of special carriers, usually various species of the Arthropoda. Thus, we now know that malaria is transmitted through the bite of infected mosquitos; African relapsing fever and Texas cattle fever, through the bite of certain infected ticks; trypanosomiasis, through biting flies. The ova of various intestinal parasites may require residence in certain of the lower animals before they can infect man.

Infection may occur along the same routes as bacterial infection, and is governed in general by the same factors of local selection, tissue susceptibility, etc. Biting insects usually deposit the parasite directly in the subcutaneous tissues or in the circulatory fluids. Abrasion of the epithelium may be necessary in order to produce infection with *Treponema pallidum*, as in the majority of the bacterial infections. The ova or larva of other parasites may be swallowed or find lodgment in the upper or lower air-passages or accessory sinuses.

It would appear that our natural defenses against infection with animal parasites are much weaker than those against bacteria. This is probably due to the greater resistance offered by animal parasites to such physical destructive influences of the host, as the acidity and germicidal activity of the secretions, temperature, etc., as well as to a general lack of natural antibodies in the body fluids of the host, and inability of leukocytes and other phagocytic cells to deal successfully with the invaders. That natural immunity against infection with certain animal parasites may exist is shown by the prevalence of certain infections among man, and their absence among lower animals, or vice versa.

The aggressiveness of animal parasites is in general probably even greater than that of most bacteria, and a more or less extensive infection apparently occurs in all cases in which the parasite had made successful invasion, some multiplying in the blood-stream (malaria, relapsing fever, trypanosomiasis, Texas fever, filariasis), others in the lymph stream (filariasis), and others in the tissues (syphilis, trichiniasis, amebiasis), without much opposition on the part of the host. Whether these factors are due to the aggressive forces of the parasites which neutralize the defenses of the host, or whether they are due to the hardness of the parasites and a lack of defense on the part of the host, is not known, but probably the latter is generally the case.

As with bacteria, animal parasites show a well-marked selective affinity for certain tissues, as the malarial plasmodium for red blood-corpuscles, trypanosomes and spirochetes for blood plasma, trichina for voluntary muscle, various parasites for the intestinal canal and even for certain portions of the intestinal tract, others for the lung, etc.

SUMMARY

From what has been said it is clear that infection differs from mere surface contamination, and cannot be said to occur until the invading parasites have reached the deeper tissues, or a point where they may grow and multiply. The surface epithelium and various secretions offer the most potent local obstacles to infection, but even when these barriers are broken down the invaders may not survive the onslaughts of various protective agencies of the host. In order to withstand and overcome these attacks the bacterium may undergo certain morphologic and physiologic changes

and actively secrete a substance that is inimical to the defensive forces of the host, or immunize itself against these forces. Thus, a certain species of bacteria may become selectively fortified or immunized against a certain host or organ of that host, and show a specific affinity for producing infection of a certain animal or a particular organ. When the bacterium has overcome the defensive forces of a host, it may, by the formation and action of exogenous and endogenous toxins, bacterial proteins, mechanical blocking of vessels, or formation of ptomains, produce disease. These various factors will be considered in greater detail in the following chapter.

CHAPTER VI

INFECTION (Continued)

PRODUCTION OF DISEASE

WHEN pathogenic parasites have reached the deeper tissues and multiplied, infection has occurred, but, as previously stated, tissue changes of sufficient extent to produce definite lesions and symptoms of disease may or may not result, depending upon whether or not the defensive forces of the host are able to overcome the invaders or are overcome by them. If the latter has occurred, and the invading parasite is firmly established in its host, the question of how the parasite and its products cause disease, that is, *the mechanism of the production of an infectious disease*, arises for consideration.

PRODUCTION OF DISEASE BY BACTERIA

The agencies by which bacteria successfully batter down the defenses of the body in the production of infection and the lesions and symptoms of an infectious disease are manifold and complex. Inflammatory changes are usually produced in a certain tissue, organ, or system of organs, and the resulting symptoms of disease depend largely upon: (1) the portal of entry, the number, virulence, and rate of multiplication of the bacterium which were discussed in the preceding chapter; (2) the production of poisonous products by the bacteria; (3) the production of toxic protein cleavage substances by proteolytic enzymes of bacteria, leukocytes, and fixed tissue cells, and (4) the degree of disturbance of physiologic function of the particular organs directly infected and those secondarily involved by circulating toxic substances.

Exotoxins and Toxinemia.—One of the most important agencies contributed by bacteria in the production of infection and disease are the soluble or exogenous toxins which they make and secrete. With some bacteria, as the diphtheria, tetanus, dysentery, and botulism bacilli, these toxins constitute the chief offensive weapon, being secreted in the infected tissues and producing local inflammation and tissue necrosis; they are also absorbed into the lymph and blood constituting a condition of *toxinemia*, and may selectively localize or affect tissues and organs remotely situated from the primary site of infection, as occurs in tetanus and diphtheria.

In addition to these typical "toxin producers," it is highly probable that the majority of bacteria, including the pyogenic cocci (staphylococci, streptococci, pneumococci, gonococci, and meningococci), the pathogenic bacilli not already mentioned (typhoid, colon, cholera, influenza, etc.), and even many saprophytic bacteria are capable of producing some of these exotoxins which exert influence in the pathogenesis of disease. It appears that these toxins possess certain properties in common, although quantitatively they vary within extreme limits, and hence in relative importance in the causation of disease.

Endotoxins.—In addition to the exotoxins it is highly probable that some of the pathogenic bacteria contain preformed intracellular toxins or endotoxins, which are liberated and become operative upon disintegration of the bacterial cells. Within recent years the existence of these endotoxins has been questioned and the toxicity of disintegrated bacteria assigned to

the presence of protein cleavage products resulting from enzymic activity or the lytic processes of amboceptors and complement. It is highly probable, however, that preformed toxins are to be found within some pathogenic bacteria and notably the pneumococcus, bound in some manner to the protoplasm which, being liberated upon dissolution of the cell, are capable of exerting an influence in the production of disease.

One possible effect of these endotoxins is in the reduction of the phagocytic activities of leukocytes; these effects have been especially studied by Bail who believes that they are caused by separate products of bacteria called *aggressins*. Whether or not they are endotoxins or separate substances cannot be definitely stated, but at any rate poisons possessing this leukotoxic action are to be found in some inflammatory exudates; doubtless they contribute a factor in the pathogenesis of disease by combating phagocytosis, which is one of the most important defenses against bacterial infection.

Bacterial Protein.—Aside from the effects produced by exotoxins and endotoxins it would appear that bacterial protein and particularly their nucleoproteins, possess pyogenic properties and may aid in the production of local inflammation and suppuration. Furthermore, toxic protein cleavage substances are probably produced by the digestion of dead and devitalized bacteria by their own proteolytic enzymes or those derived from the leukocytes, fixed tissue cells, and fluids of inflammatory exudates. In the majority of diseases the amounts of toxic split proteins derived from bacterial substrate must be small, but nevertheless are to be considered as additional factors in the pathogenesis of disease.

Furthermore, recent investigations have indicated that bacterial proteins may bring about certain obscure and unexplained physical and chemical changes in the plasma resulting in the removal of antienzymes followed by digestion of blood constituents by proteolytic enzymes with the production of protein poisons.

Toxic Exudates and Ptomaines.—Additional toxic protein substances are probably produced during some infections by digestion of constituents of inflammatory exudates by the liberated proteases of leukocytes and fixed tissue cells. These protein cleavage products are known to be highly toxic for experimental animals and when absorbed from inflammatory foci doubtless contribute in an important manner to the production of fever and other phenomena of infection.

In addition to these toxic products resulting primarily or secondarily from bacterial infection it is probable that the *mechanical action* of bacteria may sometimes be a factor of importance and especially in bacteremias of pyogenic cocci during which metastatic abscesses develop in the kidneys and other organs. In certain protozoan infections, as malaria and trypanosomiasis, embolism may play a more important rôle by blocking small, but physiologically important, vessels with masses of parasites.

Bacterial Toxemia.—The sum total of the effects of those different soluble substances produced during bacterial infection may be said to constitute *bacterial toxemia*. The term "toxemia" is frequently used for designating the effects of an exotoxin, as in tetanus, but, as mentioned above, I believe the term *toxemia* is better adapted for designating the effects of an exotoxin.

Toxemia is scarcely ever entirely absent in bacterial infections, but varies considerably in degree, being severe in such diseases as typhoid fever, influenza, pneumonia, meningitis, etc., and relatively feeble in minor pyogenic surgical infections. The general symptoms, however, are strikingly

similar whatever the variety of infection, embracing fever, changes in the pulse, quantitative and qualitative changes in the leukocytes, malaise, anorexia, muscular weakness and pains, headache, and depression.

Bacterial toxemia strictly refers to the presence in the blood and lymph of toxic products of bacterial activity. As stated above, these may be almost solely exotoxins in diphtheria and tetanus, but in other infections are due to exotoxins, liberated preformed endotoxins, and various soluble and toxic split proteins resulting from the cleavage of destroyed bacteria and, more importantly, of destroyed fixed tissue cells, and the fluid and cellular elements of inflammatory exudates.

Aside from the primary local effects produced by infection varying according to the part or organ involved, these toxic substances may produce important *secondary effects* in organs remotely situated, and particularly the nervous system, heart, spleen, and kidneys.

The production of bacterial diseases is, therefore, an exceedingly complex process in which many factors are concerned and for which simple explanations are not possible. It is true that a large amount of experimental data has accumulated, but so many technical and unexpected biologic factors enter into experiments that the subject still demands a great deal of study and investigation for the elucidation of many problems concerning infection and the production of disease.

PRODUCTION OF DISEASE BY HIGHER PLANTS

Little is definitely known of the agencies by which the microfungi produce disease. Curiously, the subject has not received much attention from mycologists, most effort being devoted to morphologic and cultural characteristics.

Some fungi, as those causing ring-worm, produce but slight inflammation, while others produce extensive suppuration, as in actinomycosis and blastomycosis. It is probable that some fungi produce exogenous toxins and that endotoxins may be liberated upon breaking up the fungi; these, in addition to toxic split protein products from fungi and body cells, are likely responsible for the local inflammatory changes and also for the symptoms of toxemia accompanying severe infections. Infections with fungi may be mixed with bacterial infection, the latter adding elements in the production of local and general reactions. A study of the toxins of microfungi and other agencies by which they produce disease offers an interesting and extensive field of investigation in mycology.

PRODUCTION OF DISEASE BY ANIMAL PARASITES

Animal parasites may produce deleterious effects in five principle ways:

1. By abstracting food material from the intestine which has not yet been assimilated. This is probably of minor importance, but may be a pathogenic factor in infestations with some of the tapeworms and especially *Tænia saginata*, which may be many yards in length.

2. By abstracting blood as by the hookworms, flukes, leeches, and blood-sucking arthropods.

3. By mechanical injury to tissues and organs. In this connection may be mentioned the blocking of blood-vessels by blood flukes, trypanosomes, and subtertian malaria protozoa; the partial or complete blocking of lymph vessels by filaria and of bile and pancreatic ducts by liver flukes.

Under this heading mention may also be made of certain parasites destructive for tissue cells, as the malarial protozoön for erythrocytes; the

pathogenic ameba which may bore into the intestinal mucosa, the lung flukes, fly maggots and trichinella, guinea-worm and itch mites producing injury by migratory and boring activities.

4. By carrying pathogenic bacteria into the tissues. This refers chiefly to *Amœba histolytica* boring into and under the intestinal mucosa carrying *Bacillus coli* and other bacteria which aid in the inflammatory processes, ulceration, and toxemia; also *Amœba gingivalis* boring deeper and deeper into the tissues of the gums, and carrying various bacteria into deeper and healthy tissue in the production of pyorrhea gingivalis.

5. By the formation of toxic substances. Comparatively little is known of this phase of the problem. Some, as, *e. g.*, *Treponema pallidum* and the spirochete of relapsing fever, probably cause disease largely through the production of toxins, especially of the intracellular variety. The chill, fever, and sweat of malaria suggest the liberation of toxic products coincident, or nearly so, with segmentation and rupture of plasmodia. The late symptoms of sleeping sickness, and the whole course of relapsing fever are strikingly similar to the bacterial toxemias. Gastel¹ has shown the presence of a toxemia in infestations with *Trichinella spiralis* and the production of toxins by other worms is possible.

Since the chief pathologic effect of some of the parasitic worms seems to be an anemia, the possible production of an hemolysin is to be considered as a partial cause of anemia in addition to hemorrhages from the bowel. Hemolysins have been repeatedly extracted from certain types of intestinal parasites. Faust and Tallqvist² believe that the hemolytic substance from an intestinal cestode (*Bothriocephalus*) is in the nature of oleic acid esters, and perhaps other fatty acids. The more recent researches of Schwartz of the Bureau of Animal Industry,³ who obtained hemolysins from *Ascaris*, *Ancylostoma*, *Trichuris*, and several other forms, seem to show, however, that different agents must be involved and that the toxic symptoms of helminthiasis are probably caused by the secretion of toxic substances. Furthermore, Beumer⁴ has recently shown that feeding oleic acid to animals is not accompanied by permanently untoward effects, which disproves the possible harm of oleic acid as a hemolytic cause of anemia.

Nevertheless, we know comparatively little of the offensive factors, and still less of the immunologic defensive factors, operative during the course of infestations with animal parasites. With the development of a technic for the cultivation of animal parasites *in vitro*, similar to that devised for the ameba, certain trypanosomes, spirochetes, and malarial plasmodia, we will be enabled to study the products of their growth or of disintegration, and the immunologic agencies concerned in infection and recovery; this offers a very important and fruitful field for research.

THE COURSE OF INFECTIOUS DISEASE

In conclusion, we may briefly consider the results of infection or the general symptoms following bacterial growth and the manner in which these are produced.

The Stages of Infection.—Practically all infections pass through the following stages:

1. *The period of incubation*, which begins at the time of infection and ends with the development of the earliest general symptoms, during which

¹ Centralbl. f. Bakteriöl., orig., 1914, 74, 254.

² Arch. f. exper. Path. u. Pharmakol., 1907, 57, 367.

³ Jour. Amer. Med. Assoc., 1920, 75, 1786.

⁴ Biochem. Ztschr., 1919, 95, 239.

time the invading parasites are multiplying in the tissues of the host. During this stage no symptoms, or only those of a purely local nature, are present. This period varies considerably in different infections, and to a lesser extent in different individuals having the same infection. Some parasites may be so virulent as to overwhelm the body cells, thus making the period of incubation very short or entirely unobservable. On the other hand, as, *e. g.*, in rabies, the period may be of several weeks' and, indeed, of several months' duration. In tuberculosis there is usually a primary local growth, which develops so gradually and the toxins are so slowly diffused that it is difficult or, indeed, impossible, to estimate the length of the period of incubation.

According to Vaughan, during the period of incubation the bacteria or their toxins or the viruses are actively engaged in changing the natural body proteins into new and specific bacterial proteins, and since this stage is constructive, there are no symptoms and the host is not ill. Even with the experimental administration of the most poisonous of toxins a definite period of incubation is usually to be observed, which cannot be reduced below a certain minimum, independent of the size of the dose injected; in general, however, a large dose of bacteria or of toxin is likely to be followed by a shorter period of incubation than if a smaller dose were administered. Similar views have been advanced by von Pirquet. In studying serum sickness, an anaphylactic phenomenon frequently observed in man following the administration of horse-serum, von Pirquet argued that the period of from eight to ten days usually following the injection before the appearance of symptoms was the time required for the production of the antibody, which then reacted upon the serum still remaining in the body cells and fluids, and that the products of this interaction caused the lesions and symptoms of serum sickness. It was then but a short step to apply these principles to infectious diseases. This "period of incubation" was formerly regarded as representing a stage during which the infecting micro-organisms multiply in the body of the infected individual, to that point at which they could give rise to symptoms of disease through the agency of their toxins or through interference with the metabolism of the host in other ways. He and Vaughan would have us believe that during this period antibody formation is taking place, and that an antibody-antigen reaction will occur with the development of pathologic changes and symptoms just as soon as these changes have progressed to a certain point. The period of incubation will vary not only in point of time of reaction but also qualitatively and quantitatively, and using this as a basis von Pirquet recognizes three main groups, depending upon whether the antibody is present in our body fluids as the result of a previously acquired infection (accidental or by vaccination), or whether it must first be developed.

Group I.—Reaction appears after eight to twelve days, as in measles, smallpox, whooping-cough, chickenpox, and other infectious diseases in which the antibodies must be developed before the symptoms are produced. If at this time the antigen, *i. e.*, either the albumins of the horse-serum, if we are dealing with serum injections, or the bacteria, in case of an infection, has disappeared from the body, no symptom will, of course, result; if, however, some of the material is still present, a reaction occurs, during which the protein poison (anaphylatoxin) is produced, and to which, in turn, the symptoms that then develop may logically be attributed.

Group II.—The reaction appears after three to seven days. If, on the other hand, the secondary infection, as, *e. g.*, pneumonia, erysipelas, etc., is acquired after a lapse of months or several years, or if the second infec-

tion of serum is given after this time, *i. e.*, at a time when the antibodies called forth by the primary infection or first injection have disappeared, a certain interval of time will elapse before symptoms of sickness develop, as in the case of the first group. This interval, however, instead of being from eight to twelve days, is now from three to seven, a fact readily explained on the basis that a cell that has once been stimulated to active antibody formation will subsequently respond to the same stimulus with increased activity. This has been called by von Pirquet the *accelerated reaction*.

Group III.—The reaction appears immediately. If the first injection of horse-serum or infection is followed by actual disease or vaccination, the reinjection or reinfection is acquired at a time when the antibodies are present in the circulation in considerable amount, a reaction will occur either immediately or within the first twenty-four hours. This reaction may be quite virulent in intensity, although it is shorter in duration than when it occurs in the first group. von Pirquet speaks of this as the *immediate reaction*. It is to be observed in cases of serum sickness where the symptoms develop almost immediately following an injection of serum months and even years after a previous injection; it also occurs in cowpox vaccination, where a local reaction takes place very quickly and soon disappears after a previous attack of smallpox or vaccination.

If the antibodies are present in lesser amounts, the reaction may occur in from the second to the fourth day; this is called the *torpid early reaction*.

At the time of the second injection of serum or reinfection with bacteria a small amount of antibody may still be present; this will give an immediate though mild reaction, and is not enough to neutralize the total amount of foreign protein introduced. A portion of the latter, therefore, will result in the production of an additional amount of antibody, which occurs in an accelerated manner, and coming in contact with some of the free antigen, gives rise to the accelerated reaction. Hence, we may have an *immediate, followed by an accelerated, reaction*.

To illustrate these principles, von Pirquet names vaccinia as an example of an acute infection in which the processes may be observed on the skin. As the result of vaccination a colony of micro-organisms is formed on the skin. For the first two days the local response is evidently traumatic in character. After the third or fourth day the specific reaction sets in, in the form of a small papular elevation surrounded by a small areola due to the local action of toxins or protein poison from disintegrated micro-organisms. By the eighth day a vesicle has formed, and from its contents new colonies can be grown on thousands of other arms. But one or two days later the ferment-like antibody appears. The colony is attacked, its contents are digested, a toxic substance is formed that diffuses into the neighboring tissues, and the intense local inflammation which we call the areola appears. In addition, the toxin enters the general circulation and fever sets in. Simultaneously the micro-organisms are destroyed, and we may no longer be able to vaccinate with the contents of the now yellow pustule. After two or three days the real struggle is ended, although the local lesion may be aggravated by secondary infection, and the body contains the new antibody for a long time.

If we now revaccinate, the antibody present will at once attack and digest the micro-organisms introduced into the scarification, and, as these do not have time to multiply, only an extremely small amount of toxin is formed which gives the "immediate or early reaction" in vaccinia. If a number of years have elapsed between the first and the second vaccination,

antibodies may be absent or present in only small amount, but the body cells have been "keyed up" by the first vaccination, and hence react more quickly to the second. The antibodies are produced in from three to five days, and attack the micro-organisms before they have had time to multiply in sufficient numbers; the relatively small amount of digestion product produces a comparatively mild local inflammation and practically no general symptoms. This is sometimes known as the "immunity reaction," or vaccinoid, and is illustrated in Fig. 178.

In a given case the period of incubation may be determined by several factors:

(a) The number of parasites gaining entrance, and especially their toxicity and aggressiveness. The primary factors are the degree of toxicity and the amount of toxic substances produced and absorbed.

(b) Upon the site of infection. Thus, the introduction of rabies virus or of tetanus bacilli into the tissues of the face or into a deep wound is likely to be followed by a shorter period of incubation than when these are introduced into the foot or in superficial wounds.

(c) Upon the degree of resistance offered by the host. For instance, one individual may contain more antitoxin or bacteriolysin for a certain bacterium than another, and consequently a longer period of incubation is required, during which these substances are neutralized and an excess of toxic bacterial substance is produced. In fact, these may offer such resistance to the bacterium that the process of infection is inhibited, or but slight and evanescent disturbances appear.

(d) Upon the general susceptibility of the host and the route of invasion.

2. The *period of prodromal symptoms*, characterized by systemic disturbances of a relatively mild type, due to diffusion of the bacteria and their products into the general circulation and their wide-spread effect upon the body cells in general. If the parasites select a special tissue or organ for attack, as the typhoid bacillus for lymphoid tissue, and pneumococci for the lungs, definite symptoms develop later, their nature depending on the special tissue or organ involved. The prodromata, however, are more marked, and indicate a wide-spread but mild action upon the body cells in general. Vaughan believes that these symptoms mark the time when sufficient proteolytic ferments have been generated by the body cells against the new bacterial protein of the invading bacteria to attack the latter, splitting the molecule and liberating a toxic moiety responsible for the general symptoms of intoxication.

3. The *period of fastigium, or of high fever*, during which the disease is at the height of its severity. Special and distinctive symptoms and lesions, according to the organ or organs especially involved, are present; the struggle between the offensive and defensive forces of parasite and host is at its height, with remissions or exacerbations dependent upon the supremacy of any one of these, and the general stability of the body cells in withstanding the wear and tear. During this time the protective proteolytic ferments of Vaughan are most active in disrupting the newly formed bacterial protein, with the liberation of the toxic portion. This process may be so active as to overwhelm the host with the toxic split product, or lead to grave secondary lesions, such as extensive necrosis, perforation of a viscus, or hemorrhage.

4. The *period of decline*, during which the patient is gradually overcoming the infection, and amelioration of the symptoms takes place.

5. The *period of convalescence* is now ushered in, during which the host gradually overcomes the effects of disease and returns to health.

During this entire time the emaciation and tissue exhaustion leave the patient quite weak, and undue exertion, errors in diet, or reinfection may lead to a *relapse*, or a reactivation of the disease. Certain *sequelæ* or morbid conditions may follow a disease, and are due to the same original cause; e. g., in typhoid fever the development of cholecystitis; at any time during the disease *complications*, or morbid conditions due to some other micro-parasite, as the development of pneumonia during the course of typhoid fever, may seriously jeopardize the life of the patient.

Grades of Infection.—According to the manner in which a parasite and its products act upon the cell of a host and the power of the host to neutralize or overcome these the following various grades and types of infection are encountered:

(a) *Malignant or fulminating infection*, during which there is no fever, but, on the contrary, a *subnormal temperature*, with rapid prostration of the patient and death within a brief period. The cells of the body are overwhelmed and paralyzed by the toxic substances; metabolism is arrested, and the heat centers are exhausted with the fall of the temperature, an indication of the intense and overwhelming intoxication.

(b) *Acute infection*, which is the ordinary type of an infectious disease as previously described, and having a definite incubation period, prodromal symptoms, fastigium, defervescence, and convalescence.

(c) *Chronic infection*, or a prolonged process characterized by insidious onset and symptoms of relatively mild or moderate severity, and terminating either in death, after months or years, or in gradual recovery. A chronic infection may be *remittent*, as may be observed in the rheumatic group of disorders; during the remission with defervescence the infecting bacterium is not totally destroyed, and subsequently lights up, producing an acute exacerbation of the disease.

In chronic infections it would appear that the parasites develop and produce their toxins slowly, or that these are slowly and imperfectly absorbed on account of the sluggish local circulation and the presence of scar tissue. The body cells become accustomed, as it were, to these toxic products, and produce only sufficient antibodies to effect their immediate neutralization. The bacteria themselves become distinctly resistant to the action of the tissues and the defensive forces, and there is neither the same degree of intoxication nor reaction as are seen in acute infections. Gradually, however, the body cells become exhausted, and unless the cells are aroused and stimulated by judicious administration of bacterial vaccines to produce an oversupply of antibodies, the host shows progressive emaciation and weakness.

The Systemic Reaction to Infection.—It is not within the scope of this book to discuss the various symptoms of infection, and we will limit ourselves to a brief discussion of the most important, namely, the febrile reaction.

According to Vaughan, the fever of infection is due mainly to the toxic split protein resulting from the action of the protective proteolytic ferments upon the new bacterial protein. This observer and his associates were able, by the injection of multiple doses of protein derived not only from the typhoid bacillus but from various vegetable and animal proteins, to reproduce experimentally in rabbits a febrile reaction known as *protein fever*, and which is not unlike typhoid fever. This induced fever may continue for weeks, and is accompanied by increased nitrogen elimination and gradual wasting; it is followed by immunity, and the serum of immunized animals digests the homologous protein *in vitro*. As has repeatedly been

stated, Vaughan regards the split toxic product as the cause of the general symptoms of infection, the special and characteristic symptoms and lesions of the different diseases depending upon the site where the bacterial proteins have been deposited, and where they are, in large part at least, digested.

In addition to this toxic action of split protein, fever may be due: (a) to the unusual activity of the cells supplying the proteolytic enzymes, and (b) to the cleavage of the foreign bacterial protein by these ferments.

The fever of infection, therefore, is caused by the toxic action of pathogenic parasites, both bacterial and animal forms, upon the body cells and heat-regulating centers. It must be regarded by itself as a beneficent phenomenon, inasmuch as it marks a reaction of the body cells to toxic agents, for the purpose of neutralizing these and, by the development of antibodies, ridding the body of foreign substances.

BACTERIAL TOXINS

Nomenclature.—Of all the various means whereby bacteria produce disease, none possesses so much importance as the poisonous substances known as *toxins*, elaborated by the metabolic activities of the micro-organisms. A few classes of bacteria secrete this poisonous principle directly into the tissues or artificial culture-media in which they are growing, and hence are known as *soluble*, *exogenous*, *extracellular*, or *true toxins*. Other bacteria retain most of their toxins within the bacterial cell, and for this reason are called *endotoxins*, or *intracellular toxins*; these are liberated upon the disintegration of the bacteria by various mechanical, physical, or chemical means.

By common consent the term "toxin" is applied to the soluble or true toxins, such as those of diphtheria and tetanus, and hence the term, when used without further qualifications, may be considered to refer to toxins of this class.

Aside from bacterial toxins, characteristic poisons are also produced by certain of the higher plants (phytotoxins) and animals (zoötoxins), and although few are of medical interest, their study has thrown considerable light on the phenomena of toxin-antitoxin immunity.

EXTRACELLULAR BACTERIAL TOXINS

Definition.—*Bacterial toxins may be defined as poisonous products produced by bacteria in both living tissues and artificial culture-media.* The symptoms resulting from their activity appear after a certain period of incubation, and all are capable of stimulating the production of specific antitoxins. They represent the chief poisonous product of bacteria, and are mainly responsible for the symptoms of infection caused by the specific bacteria that have produced them. The first to be studied was diphtheria toxin by Roux and Yersin¹ in 1888 and 1889. The methods adopted by these investigators enabled others to discover analogous toxins of several other bacteria. Faber² and Brieger and Fränkel³ soon succeeded in separating the toxin from the tetanus bacillus, a toxin capable of producing in animals tetanic contractions as typical as those obtained with cultures of the tetanus bacillus.

¹ Ann. d. l'Inst. Pasteur, 1888, 2, 629; *ibid.*, 1889, 3, 273.

² Berl. klin. Wchnschr., 1890, 717.

³ Berl. klin. Wchnschr., 1890, No. 11.

The true toxins causing infection in man are chiefly:

1. Diphtheria toxin.
2. Tetanus toxin.
3. Botulism toxin (a form of meat poisoning).
4. Dysentery toxin (Kruse-Shiga).
5. Staphylotoxin, streptotoxin, *Bacillus welchii* toxin, and other bacterial toxins.

General Properties of Soluble Toxins.—Many of the true toxins are extremely labile, and susceptible to the action of heat, light, age, etc.; consequently an absolutely pure toxin is practically unknown. Most bacterial toxins are apparently destroyed by heating at 58° to 65° C.; Landsteiner and von Rauchenbichler,¹ Dreyer and Blake,² however, believe that at this temperature the toxins of staphylococci and *Bacillus megaterium* are masked by union with other substances, but not actually destroyed inasmuch as additional exposure to 100° C. for ten minutes or more restores toxicity. Oxygen, even as it occurs in the air, is harmful; all oxidizing agents, including the oxidizing enzymes, quickly destroy them, and Pitini³ has ascribed the harmful effects of toxins to their power of reducing the oxidizing capacity of the tissues. Some substances seem to attack only the toxophore portion of the toxin molecule, *e. g.*, iodine and carbon disulphid (Ehrlich). In the preparation of antitoxin the first doses of toxin are frequently modified by adding a chemical of this nature. According to Gerhartz⁴ x-rays tend to weaken the toxins.

Because of their great lability the toxins do not lend themselves to accurate chemical analysis. Our knowledge of them has been gained largely through a study of the lesions and symptoms produced by injecting the toxins into susceptible animals.

They are, so far as known, uncrystallizable and thereby differ from ptomaines; they are soluble in water and dialyzable through thin but not thick membranes. They are precipitated along with peptones by alcohol and also by ammonium sulphate.

The toxins are all poisonous, but in order to exert their toxic effect they must enter into chemical combination with cells; hence there is a necessary period of incubation before symptoms of their activity appear. Most bacterial toxins are not absorbed from the intestine (botulinus toxin excepted), and when introduced into the gastro-intestinal tract they are usually unable to produce symptoms and are quickly destroyed.

An essential property of a toxin lies in the fact that we can immunize a subject against it, and are able to demonstrate the presence of antitoxin within the serum of the immunized animal.

Chemical Properties of Exotoxins.—As has just been stated, the exact chemical nature of toxins is unknown. This is due principally to the fact that pure toxins of bacteria are rarely obtainable except in conjunction with their associated products, such as lysins, pigments, acids, etc., as well as to the great lability of the toxins. A summary of the results of researches into the chemical nature of toxins would indicate that they are toxalbumins, albumoses, or allied to the albumoses. Certain investigators have reported that very active toxins obtained by purification processes did not give the protein reactions, yet toxins are digested by proteolytic ferments, and, like proteins, are precipitated by nucleic acid (Kossel). Ac-

¹ Ztschr. f. Immunitätsf., orig., 1908, 1, 439.

² Lancet, 1904, 2, 409.

³ Biochem. Zeit., 1910, 25, 257.

⁴ Berl. klin. Woch., 1909, 46, 1800.

cording to Field and Teague,¹ the toxins act like electropositive colloids, but diffuse faster than do proteins. Our present knowledge of the chemistry of the true toxins has been expressed thus by Oppenheimer: "We must be contented to assume that they are large molecular complexes, probably related to the proteins, corresponding to them in certain properties, but standing even nearer to the equally mysterious enzymes with whose properties they show the most extended analogies both in their reactions and in their activities." Warden, Connell, and Holly,² in a recent study of the nature of the toxins and antigenic activities of *Bacillus diphtheriæ* and *B. megaterium*, concluded that the lysins and toxins of these two micro-organisms were the same substances, being respectively the specific fat antigens existing in definite and particular colloidal states.

Precipitation of the Exotoxins.—After the toxin has been secured by filtration, crystals of ammonium sulphate are added in large excess over the saturation point, and the whole kept at 37° C. for eighteen hours. The toxin is precipitated and rises to the surface along with the albumoses and peptones. This is skimmed off and quickly dried with an electric fan and cold air. The residue is ground into a fine powder and stored in vacuum tubes kept at a low temperature and in a dark place. During this process there may be considerable deterioration and especially with tetanus toxin. Banzhaf has obtained highly potent and dried diphtheria toxin by slightly acidulating the toxin broth and adding absolute ethyl alcohol up to 65 per cent.; after an hour or two the slight precipitate is filtered off, quickly dried, and kept in ampules.

Structure of Exotoxins.—According to Ehrlich, the toxin molecule consists of a main central atom or radical, with a large number of organic side chains grouped, as in other organic compounds, about this main radical. Each of the side or lateral arms is composed of two portions—one, the haptophore group, which has a chemical affinity for certain chemical constituents of the tissues of susceptible animals, and the other, the injury-producing portion, called the toxophore group (Fig. 40). An animal is susceptible to a toxin only when its cells contain substances that possess a chemical affinity for the haptophore group of the toxin, and also substances susceptible to the toxic action of the toxophore group.

The toxophore group is far more unstable and susceptible to deleterious influences than is the haptophore portion. When the molecule has lost the toxophore radical it is known as a *toxoid*, which is still capable of uniting with the side arms of cells, but is devoid of toxic action.

Nature of Exotoxins.—It has been abundantly demonstrated that toxins are colloids, and in many respects bear a close resemblance to enzymes. The toxins are synthetic products of bacterial activity. They are of absolutely specific nature, and in this manner differ from ptomains, which are cleavage products from the medium upon which the bacteria have been grown. Furthermore, ptomains of similar properties may be produced by several different kinds of bacteria, and accordingly are non-specific in nature. Toxins, like ferments, can give rise to antibodies, whereas ptomains cannot produce them.

The extracellular or soluble toxins differ from the intracellular toxins in that they are more easily diffused throughout the animal juices, and that their diffusion occurs independently of the invasiveness of the bacteria, so that comparatively few micro-organisms growing at some unimportant focus and causing but slight local lesions may be able to give rise to pro-

¹ Jour. Exper. Med., 1907, 9, 86.

² Jour. Bacteriology, 1921, 6, 103.

found general intoxication. This is well illustrated in diphtheria, where the local lesion in the throat may be quite small, and in tetanus, where it may indeed be undiscoverable—yet either, through the action of their toxins on special tissues, may cause profound intoxication and death.

Similarity Between Toxins and Ferments.—The toxins bear a well-recognized and close resemblance to the organic ferments; these points of resemblance may be summarized as follows:

1. Both toxins and ferments are products of the metabolism of living animal and vegetable cells, and may be extracellular (free enzymes and soluble toxins) or intracellular (intracellular enzymes and endotoxins).

2. Both are colloids; both pass through porcelain filters and are largely held back by dialyzing membranes.

3. Both are usually affected by temperatures above 70° C., the toxins slightly more, however, than the ferments. Most toxins and ferments are destroyed at 80° C. In solutions both toxins and ferments deteriorate with the production of toxoids and fermentoids.

4. The activities of both toxins and ferments seem to depend largely upon the temperature to which they are exposed.

5. Both exhibit a latent period before manifesting their individual activities; in general, the effect of each is more rapid the larger the amount present. Both are poisonous for animals and when injected produce antibodies.

6. Both substances represent a method or means by which the organism attempts to modify its environment and render the surroundings suitable for nutrition and growth.

7. Both show a strong affinity of their substratum, and first manifest their activity by combining with it. For example, fibrin placed in gastric juice at 0° C., and then repeatedly washed in cold water to remove all traces of pepsin will undergo digestion when raised to body temperature. Similarly, if red corpuscles are placed in fresh tetanus toxin at 0° C. for an hour, washed repeatedly with cold normal saline solution, and then raised to 37° C., hemolysis will take place, indicating the primary union of the bacterial hemolysin or tetanolysin with the corpuscles. In a similar manner toxins probably unite chemically with tissue cells, as the toxin quickly disappears from the blood following its injection and but a small fraction can be recovered from the excretions. Furthermore, the injection of an emulsion of these cells into other animals may be followed by specific symptoms of intoxication.

8. The one great difference, however, between toxins and enzymes is the greater activity of the latter, even very minute amounts of an enzyme having the power to split up or decompose large quantities of complex organic compounds. An enzyme attaches itself to a substance and absorbs water; the molecule breaks down, the enzyme is liberated, and then attacks another molecule, this process being repeated until large amounts of fermentable substances have been attacked. When, however, a toxin has united with a substance it loses its identity and in this manner it follows the law of multiple proportions. This has been discussed as it relates to the soluble toxins of diphtheria and tetanus, and is likewise easily demonstrable in the action of tetanolysin upon erythrocytes of the rabbit. It is true that a toxin may become dissociated and attack another molecule, but this action is different from that of an enzyme, because the molecule first attacked is not injured. However, as Adami points out, the toxins may be equally active in the body until arrested by antitoxins, although experiments *in vitro* clearly demonstrate the greater activity of the ferments.

Von Liebermann¹ denies the identity of toxins and ferments on the basis of experiments with ricin, because this substance appears to be "used up" in the agglutination of erythrocytes, is not appreciably affected by hydrocyanic acid, and is more thermostabile than toxins. Coca,² on the other hand, believes that the toxin of cobra venom is a ferment in the nature of a lipase and subscribes to the view that toxins are ferments. Both of these investigators, however, worked with non-bacterial toxins and absolutely conclusive evidence that toxins are ferments has not been produced, largely because it is practically impossible to isolate either in an absolutely pure form for experiments. As bearing upon the relation of bacterial toxins to ferments mention may be made of the experiments of Dr. Moshage and myself,³ showing that toxin production by diphtheria bacilli is not absolutely parallel with the production of carbohydrate-splitting ferments, although these ferments were produced most frequently and vigorously by the best toxin-producing bacilli.

Selective Action of Exotoxins.—Extensive studies of the toxins of diphtheria and tetanus and of cobra venom have shown that they are quite complex, and are usually composed of two or more distinct and separate toxins possessing different pathogenic properties, although one of these may predominate in producing symptoms.

All infections with the group of true toxic-producing bacteria manifest certain non-specific symptoms of general intoxication, namely, fever, headache, malaise, prostration, etc.; but the typical symptoms of these diseases are due to the remarkable selective action of the toxins upon certain cells or organs, dependent upon the ability, chemical, physical, or both, of the toxin to combine with these specific cells. For example, tetanus toxin contains *tetanospasmin*, that has a special affinity for nervous tissue; and *tetanolyisin*, a poison that has a selective affinity for erythrocytes and is hemotoxic. Ehrlich has shown that these are really different toxins, and not one toxin with a twofold function, even the antitoxins of the two being different. Similarly, the general symptoms and necroses of diphtheria are attributed to the main toxin of the bacillus, and the nerve lesions and paralyses to a secondary but distinct secretory product known as *toxon*. This latter view of Ehrlich's, however, is much disputed, many investigators believing that toxon represents a degenerated or modified form of the one toxin.

Wadsworth and Vories⁴ have recently shown that neither the leukocytes of the dog nor those of the guinea-pig neutralize or combine with diphtheria or tetanus toxin. Although tissue combines with and neutralizes tetanus toxin it has no action on diphtheria toxin.

The special affinities of toxins for certain tissues have analogies among the poisons of higher plant life, as, for example, strychnin has a similar selective affinity and is said to be specific in its action upon the motor cells.

The venom of various serpents, especially that of the cobra, has specific action; the erythrocytes of various animals are readily attacked by it, and the cells of the respiratory center are apparently profoundly affected.

Aside from the special effects of the toxins upon certain cells and tissues, it must be remembered that toxins may involve the body cells in general, and particularly those of the parenchymatous organs, such as the kidneys, heart, and liver, causing coagulation of the protoplasm (cloudy swelling)

¹ Deutsch. med. Wchnschr., 1905, 31, 1301.

² Jour. Infect. Dis., 1915, 17, 351.

³ Jour. Infect. Dis., 1916, 19, 28.

⁴ Jour. Immunology, 1921, 6, 413.



FIG. 52.—ABDOMINAL WALL OF GUINEA-PIG SHOWING DIPHTHERIC EDEMA.

Shows abdominal wall of a guinea-pig forty-eight hours after subcutaneous injection with 2 c.c. of a seventy-two-hour bouillon culture of a diphtheria bacillus isolated from the throat of a diphtheria convalescent.

and final dissolution. The harm brought about by the toxins or toxic products of the pyogenic group of micro-organisms, for instance, acts mainly in this manner.

SPECIAL PROPERTIES OF THE PRINCIPAL TOXINS

1. Diphtheria Toxin.—Diphtheria bacilli vary considerably, both in tissues and in artificial culture-media, in the quantity of toxin secreted; thus, in bouillon large amounts are seldom found in less than from seven to fourteen days.

The action of the toxin is dependent upon the dosage, and a certain period of time must always elapse before the symptoms appear, the minimum being about one day. Large doses may shorten this period of incubation, but cannot diminish it below a certain limit.

The lesion of diphtheria is practically always local, and is usually situated on the mucous membrane of the upper air-passages. It is characterized by the formation of a pearly white membrane that is adherent to the underlying edematous tissues. The toxin produces necrosis of the surface epithelium, and the product, together with fibrin and leukocytes, constitutes the membranous exudate. From this focus toxin is absorbed by the lymphatics and blood-stream, and distributed throughout the body, the bacilli being rarely found in the blood or internal organs. Later, the effects of toxin intoxication are shown by paralyses of certain motor nerves and ganglia, particularly those of the palate and heart.

When a guinea-pig receives a subcutaneous inoculation with diphtheria toxin, a typical hemorrhagic gelatinous edema develops at the site of inoculation (Fig. 52). Upon opening the abdominal cavity one finds but little peritoneal exudate, but the vessels of the mesentery are injected and the adrenal glands show characteristic acute hyperemia (Figs. 53 and 54). Bloody pericardial and pleural exudates will be found in the thorax and solidified areas in the lungs. Guinea-pigs surviving a dose of toxin may, after two or four weeks, begin to show paralysis of the hind and then of the fore extremities, a condition analogous to the postdiphtheric paralysis occurring in man and ascribed to the effects of toxin.

Method of Testing the Virulence and Toxicity of Diphtheria Bacilli.—Young guinea-pigs weighing from 250 to 300 grams are quite susceptible to diphtheria toxin, and are used in determining the strength of a toxin and in standardizing antitoxin. The test may be of great value in the management of convalescent and "carrier" cases of diphtheria, harboring bacilli in the upper air-passages, in determining whether the micro-organisms are dangerous or merely harmless non-pathogenic saprophytes. It is practically impossible, from the morphology of the organism alone, to decide whether or not a given culture is dangerous, and prolonged quarantine may not only be irksome and inconvenient, but, if the organisms are proved to be harmless, it is unnecessary as well.

To be reliable, however, such a test must be carried out very carefully. In the case of a highly virulent culture the mere introduction of a few organisms beneath the skin will suffice to demonstrate their dangerous character, but with cultures only slightly virulent more care is necessary, for although the patient may show no ill effects as a result of the presence of the bacilli, in the throat of another and less immune individual they may be highly dangerous.

The following method has been used by the author in many hundreds of such tests, and has proved of distinct value:

1. Make a culture of the part harboring the bacilli on a tube of Löffler serum medium. Incubate at 35° C. for from eighteen to twenty-four hours; prepare a smear and stain with Löffler's methylene-blue. If diphtheria bacilli are present they must be isolated in pure culture. *Never attempt a guinea-pig test with an impure culture!*

2. Isolate by the "streak" method on plates of blood-serum.

3. Inoculate a tube of 1 per cent. glucose bouillon, which is neutral or slightly alkaline, with several different colonies.

4. Incubate at 35° C. for three days, keeping the tube in a slanted position in order to give the culture as much oxygen as possible. If a good growth does not appear in twenty-four hours, transplant to another tube of bouillon until the bacilli have been "educated" to grow on the medium.

5. Examine for purity. Select a 250- to 300-gram guinea-pig and inject 2 c.c. of the unfiltered culture in the median abdominal line. Animals over the weight specified are more resistant and less reliable for the test. The unfiltered culture is used, since toxin is but one element of the disease-producing power of diphtheria bacilli, and toxin production in bouillon may not be a true index of the toxin production in mucous membranes.

6. Carefully observe the animal for at least four days. Even slight toxemia, especially if accompanied by edema at the site of injection, should be regarded as a positive result (Fig. 52).

7. After death perform a careful autopsy. Make cultures of the edematous area, peritoneum, and heart blood. Diphtheria bacilli may be found in the edematous fluid, but will rarely be found in the peritoneum or in the blood. Observe whether acute hyperemia of the suprarenal glands is present (Figs. 53 and 54).

8. Not infrequently animals showing mild or even an absence of the symptoms of toxemia develop paralysis of the hindquarters two or three weeks later. According to Ehrlich, this paralysis is due to the action of "toxoin," a toxic substance secreted by the bacillus or, as believed by others, a modified form of toxin.

9. To prove that diphtheria was the cause of the toxemia or death mix 2 c.c. of the culture in a test-tube with 1 c.c. of diphtheria antitoxin (500 units). After standing aside for an hour at room temperature, inject the mixture subcutaneously in the median abdominal line of a 250- to 300-gram guinea-pig. Symptoms of toxemia do not develop.

In a comparative study of the above and other methods Kolmer and Moshage¹ found that washing off a pure culture from a slant of Löffler's blood-serum media with 10 c.c. of sterile salt solution, emulsifying and injecting 4 c.c. subcutaneously in the median abdominal line of a 250- to 300-gram guinea-pig, yielded equally delicate results. The intracutaneous method of Neisser, which has also been advocated by Zingher and Soletsky,² while being more economical, in that 2 pigs suffice for 4 or even 6 tests, was found to yield somewhat indefinite reactions with bacilli of low virulence.

Standardizing Diphtheria Toxin.—The strength of a diphtheria toxin is estimated by injecting subcutaneously a series of guinea-pigs weighing approximately 250 grams, with decreasing amounts of toxin. How many dilutions will be necessary it is impossible to state; for exact results several pigs of the same weight should be inoculated with the same dose, and the effects should show various gradations, dependent upon the size of the successive doses. In order to obtain a uniform method for estimating the strength of a diphtheria toxin and thus obtain comparative values, a *standard unit* has been adopted, consisting of the smallest amount of toxin that will kill a healthy guinea-pig weighing about 250 grams in from four to five days. This is known as the *minimum lethal dose*, or *dosis lethalis minimus*. The technic used for determining this dose is given in the chapter on Antitoxins.

A quick and accurate method for estimating the amount of diphtheria toxin present in the body fluids of a diphtheric patient would be of value in controlling the antitoxin treatment of this infection. At present the amount of antitoxin administered is regulated according to the clinical condition of the patient. Uffenheimer has used a method for determining the presence of toxin, consisting in injecting intraperitoneally a 250-gram guinea-pig with 0.1 to 0.4 c.c. of the patient's serum, diluted with 2 to 4

¹ Jour. Infect. Dis., 1916, 19, 1.

² Jour. Infect. Dis., 1915, 17, 454.



FIG. 53.—NORMAL ADRENAL GLAND OF A GUINEA-PIG.



FIG. 54.—ADRENAL GLAND OF A GUINEA-PIG AFTER FATAL DIPHThERIC INTOXICATION.

c.c. of salt solution. The presence of a distinct doughy edema of the abdominal cavity after seventeen to twenty-four hours indicates the presence of diphtheria toxin, an observation that may be confirmed by making an autopsy at the end of forty-eight hours. The diagnostic value of this method has not been adequately established; it is doubtful if it yields any information other than is more readily gained by making a good cultural examination of the patient, and it does not aid in the estimation of the quantity of toxin, which is the result most desired.

Diphtheria toxins have been classified into three groups, depending upon the degree of avidity for antitoxin they display, viz., prototoxin, deuterotoxin, and tritotoxin. Each of these toxin groups may, in whole or in part, be converted into toxoids. The prototoxin has a greater affinity for the antitoxin than has the deuterotoxin, and the deuterotoxin has a greater affinity for the antitoxin than has the tritotoxin. The same relation is apparent with the three toxoids, which are not poisonous, but which have the same power of combining with antitoxin as have the toxins from which they take their origin.

In standardizing antitoxin, it is found in general that with a perfectly fresh toxin a certain amount of antitoxin will just neutralize a definite amount of toxin. If older toxin is used, it is found that the toxin has lost about one-half its toxic power, but retains its initial power for neutralizing antitoxin. Ehrlich explained this by showing that the diphtheria toxin molecule is composed of two groups—one the carrier of the toxic qualities, the toxophore group, which is quite labile; the other uniting the whole molecule with antitoxin, being capable of neutralizing it, and characterized by its stability. The toxophore group being destroyed as in old toxin, the poison loses its toxic qualities, but retains its power to bind antitoxin. This modified toxin or non-poisonous diphtheria toxin has been designated by Ehrlich *diphtheria toxoid*.

2. Tetanus Toxin.—Of all bacteria classed as true toxin producers, none possesses greater toxicity than does the tetanus bacillus. The number of organisms producing sufficient toxin to cause a fatal infection may be so small that careful anaërobic cultures made from the local lesion of infection, together with injection of the wound secretions into white mice, may fail to disclose the presence of tetanus bacilli.

According to Ehrlich, tetanus toxin is composed of two separate and distinct substances: (1) *Tetanospasmin*, a neurotoxin, which is very labile and responsible for the severe symptoms of the infection; (2) *tetanolysin*, a hemotoxin, which is more stable and destructive for erythrocytes.

Tetanus toxin is prepared by cultivating the bacillus in bouillon under strict anaërobic conditions. Since tetanospasmin is so susceptible to the influence of heat, age, and even light, the toxin is best preserved in a dry form. The standard of tetanus toxin consists of 100 minimal lethal doses of a precipitated and dried toxin, preserved at the Hygienic Laboratory of the Public Health and Marine Hospital Service.

If susceptible animals, such as mice or guinea-pigs, are injected subcutaneously or intravenously with tetanus toxin, they begin to manifest symptoms after a certain period; these are due to the action of tetanospasmin upon motor nerve-cells, and are characterized by hypersensitiveness, clonic convulsions, and rigidity of the muscles. In man the symptoms of tetanus are similar to those in the animal, the spasm starting quite regularly in the muscles of the lower jaw.

Experiments by Wassermann and Takaki have demonstrated that an especially close affinity exists between tetanus toxin and certain struc-

tures, particularly that of the central nervous system. Most writers agree that the toxin reaches these tissues largely by way of the nerve paths.

Teale¹ believes that some toxin may ascend to the central nervous system by way of the axis-cylinders of the nerves, also, and to a greater extent, along the perineural lymphatics. According to his experiments the toxin does not pass through the choroid plexus into the cerebrospinal fluid. Peterson² has found that the toxin is absorbed not only by nerve-cells but also by the leukocytes and fixed tissue cells of several organs of different animals including the dog, rabbit, and guinea-pig.

3. Botulism Toxin.—This poison is generated by the *Bacillus botulinus*, first isolated by Van Ermengem in 1896 from a ham during an epidemic of meat poisoning. It is the cause of a type of meat and sausage poisoning called botulism, more frequent in those countries where raw meat is eaten, and frequently confused with "ptomain poisoning."

The bacillus is a motile, spore-forming, anaërobic bacterium, which grows at room temperature and causes marked gas formation in glucose media.

The toxin is readily produced in anaërobic alkaline bouillon cultures. It is quite labile. As shown by Edmondson, Giltner, and Thom³ different cultures vary in toxin production and both bacilli and toxin-free spores are virulent for guinea-pigs; according to Shippen⁴ the bacilli will grow and produce toxin in aerobic cultures in symbiosis with staphylococci.

Symptoms of botulism appear only after a definite period of incubation, which varies from twenty-four to forty-eight hours. In contradistinction to the meat poisonings produced by other organisms, those due to *Bacillus botulinus* may show few or no symptoms directly referable to the intestinal tract, the chief symptoms being due to toxic interference with the cranial nerves: loss of accommodation, ptosis, dilated pupils, aphonia, dysphagia, and hypersecretion of mucus from the mouth and nose.

Sporadic toxemic-like disease in cattle sometimes designated as "forage poisoning" has occurred with varying severity throughout the Middle Western states; Graham and Schwarze⁵ have observed outbreaks in equines which were quite definitely related to the consumption of feed containing *Bacillus botulinus* toxin, an anërobic bacillus biologically resembling *B. botulinus* (Type B) being isolated from a corn silage.

Guinea-pigs are quite susceptible, and may be infected by way of the mouth. The symptoms of intoxication usually follow in twenty-four hours, and are characterized by motor paralysis, dyspnea, and hypersecretion of mucus from the nose and mouth.

4. Dysentery Toxin.—The distinct types of dysentery bacilli vary exceedingly in their powers to produce toxins, the strongest poisons being produced with bacilli of the Shiga-Kruse variety, less regularly active ones, with bacilli of the Flexner type.

Investigations have shown quite conclusively that dysentery itself is a true toxemia, its symptoms being referable to the absorption of the toxins of the bacillus from the intestine. Flexner, who has studied this subject with great care, believes it probable that most of the pathologic lesions occurring in the intestinal canal are referable to the excretion of dysentery toxin rather than to the direct local action of the bacilli. The action of

¹ Jour. Path. and Bact., 1919, 23, 50.

² Ztsch. f. Immunitätsf., orig., 1910, 8, 498.

³ Archiv. Int. Med., 1920, 26, 357.

⁴ Archiv. Int. Med., 1919, 23, 346.

⁵ Jour. Bacteriology, 1921, 6, 69.

the dysentery toxin upon animals is very characteristic, and throws much light upon the disease in man. Intravenous injection of the toxin in rabbits is followed by marked diarrhea, rapid fall in temperature, respiratory embarrassment, and terminal paralysis. Upon autopsy the intestinal mucosa, especially that of the cecum and colon, shows marked inflammatory involvement, supporting Flexner's observation of the necrotic action of excreted toxin.

Dysentery bacilli also produce an endotoxin, and poisonous substances are easily obtained by extracting the bacilli themselves or by filtration of properly prepared bouillon cultures. The toxin is fairly stable, and well preserved under toluol in the refrigerator.

As shown by Olitsky and Kligler¹ the Shiga dysentery bacillus produces a true exotoxin possessing a marked affinity for nervous tissue and producing muscular weakness and paralysis of rabbits, and an endotoxin, responsible for the production of intestinal lesions. Thjøtta² has recently grouped dysentery bacilli into three groups, the Shiga bacillus being toxic and belonging to Group I; the atoxic bacilli, as the Flexner, Strong, and Hiss Y strains, were placed in Group II, while Group III embraces a second toxic strain similar to Group I.

5. Staphylococcus Toxins.—Two definite toxins have been isolated from cultures of *Staphylococcus pyogenes aureus* and *albus*, one of which exerts a destructive action on erythrocytes (hemotoxin), and the other on leukocytes (leukocidin).

An antihemotoxin that counteracts the effects of the toxin may be produced experimentally, and in human staphylococcus infections the demonstration of such antihemotoxic substances in the blood-serum may be of aid in making the diagnosis of staphylococcus infections. This anti-staphylolysin may be found normally in small amounts in the serum of man and horse, and when antihemotoxic tests with human serum are made a normal control should always be included. Antileukocidins have also been produced, but are not of practical importance.

The hemotoxin is readily formed in cultures of staphylococci; roughly, the amount produced depends upon the virulence of the culture. In human cases of staphylococcus infections this toxin produces hemolysis *in vivo*, and is partly responsible for the grave anemia that is frequently present. Orcutt and Howe³ have recently identified a staphylolysin as a fatty acid or soap formed by the action of a lipase elaborated by the cocci when cultivated in the presence of fat.

6. Streptococcus Toxins.—The grave systemic symptoms that so frequently accompany slight streptococcus lesions are strong indications that these micro-organisms produce a powerful diffusible poison, although extensive researches into the nature of these poisons have not given us any clear understanding of the subject.

Streptococci may yield soluble toxins that, when administered to guinea-pigs, produce rapid collapse and death. While these toxins are not comparable in potency to the soluble toxins of diphtheria and tetanus, they have, nevertheless, been differentiated from the endotoxins contained within the cell-bodies, and have been found to possess less toxicity.

Beside these toxins, some streptococci produce a hemolysin first described by Bordet⁴ which may be conveniently observed by cultivation of

¹ Jour. Exper. Med., 1920, 31, 19.

² Jour. Bacteriology, 1919, 4, 355; *ibid.*, 1921, 6, 501.

³ Jour. Exper. Med., 1922, 35, 409.

⁴ Ann. d. l'Inst. Pasteur, 1901, 15, 880.

the organisms upon blood-agar plates. This hemotoxin is partly responsible for the sanguineous character of a streptococcus exudate. Nakayama,¹ in a recent study of these streptococcus poisons, identified one as a leukocidin, an apparently different poison from the hemolytic toxin or streptolysin. Ruediger² failed to produce specific serum antilysins for the hemotoxin.

Gas Bacillus and Other Bacterial Toxins.—During the World War many wounds showed the presence of gas-producing and spore-forming anaërobes which received special attention to determine their rôle in wound infections and from the standpoint of development of specific serum therapy. Three of these spore-forming bacilli were found especially important, namely, *Bacillus tetani*, *B. welchii* (*B. aërogenes capsulatus*, *B. perfringens*), and *B. edematis maligni* (bacillus of malignant edema, vibrion septique).

Bull and Pritchett,³ De Kruif and Bollman⁴ have studied the toxin of *Bacillus welchii* with particular care, finding that most strains produce small amounts of a true toxin in special media which is toxic for the pigeon, producing necrosis of muscle. The presence of necrotic tissue in wounds favors the growth of these bacilli and toxin production; Bull and Pritchett succeeded in manufacturing an antitoxin for the toxin of *B. welchii* which possessed prophylactic and curative properties in experimental infections. Further reference to the results observed in the prophylaxis and treatment of wounds is given in the chapter on Serum Therapy.

Exogenous or soluble toxins have also been found by Haslam and Lumb⁵ to be produced by *Bacillus chauveau* (blackleg); Parker has described a toxin for *B. influenza*, and Zinsser⁶ believes that many pathogenic and even non-pathogenic Gram-positive and Gram-negative micro-organisms, as streptococci, influenza bacilli, typhoid colon, and dysentery bacilli, *B. prodigiosus*, *Staphylococcus aureus* and meningococci, may, under favorable conditions, produce small amounts of what appears to be true soluble toxins, although these have not been definitely proved not to be endotoxins.

Bacterial Hemagglutinins and Hemolysins or Hemotoxins.—Aside from the exogenous poisons mentioned above, mention has been frequently made of hemolytic poisons produced by some pathogenic and saprophytic bacteria, notably *Bacillus tetani*, *B. welchii*, staphylococci, and streptococci. Other micro-organisms may contain an endohemotoxin, as the pneumococcus, capable of converting hemoglobin into methemoglobin.

All of these hemotoxins possess an affinity for erythrocytes and act upon them directly; antisera have been produced for some of them capable of neutralizing their effects, but it is not certain that this may be partly due to serum cholesterol.

Some bacteria may produce agglutinins for human erythrocytes, among them being the staphylococcus, *Bacillus typhosus*, and *B. pyocyaneus*; these have not been studied as thoroughly as the hemotoxins.

These hemolytic poisons are probably responsible in whole or part for the secondary anemias occurring in the infectious diseases and particularly those accompanied by septicemia; they may also produce hemoglobinuria. Connell and Holly⁷ believe that they consist of bacterial fats in definite colloidal states and further reference to them will be made in Chapter XX.

¹ Jour. Infect. Dis., 1920, 27, 86.

² Jour. Infect. Dis., 1907, 4, 277.

³ Jour. Exper. Med., 1917, 26, 119.

⁴ Jour. Infect. Dis., 1917, 21, 588.

⁵ Jour. Infect. Dis., 1919, 24, 362.

⁶ Jour. Immunology, 1920, 5, 265.

⁷ Jour. Bacteriology, 1921, 6, 89.

TOXINS OF MICROFUNGI AND HIGHER PLANTS (PHYTOTOXINS)

As previously mentioned, the power of forming toxins is not confined to bacteria alone. It is well known that certain plants produce soluble toxins which may be operative in the production of disease, and it is highly probable that some of the pathogenic microfungi may do likewise.

For example, the poisons of *Rhus toxicodendron* (poison ivy and oak) and *Rhus venenata* (sumac and dogwood) are well known to produce a form of dermatitis designated as dermatitis venenata. Some persons are extremely susceptible to these poisons suggesting hypersensitiveness or an anaphylaxis to the proteins of the poisons; this subject is discussed at greater length in Chapter XXVIII.

The best known phytotoxins are as follows: *Ricin* (from the castor bean, *Ricinus communis*); *crotin* (from the seeds of *Croton tiglium*); *abrin* (from the seeds of *Abrus precatorius*); *robin* (from the leaves and bark of *Robinia pseudoacacia*); *curcin* (from the seeds of *Jatropha curcus*), and the pollens of a large variety of grasses and plants (ragweed, goldenrod, etc.). A large number of poisonous mushrooms are also known to contain poisons—*phallin* (from *Amanita phalloides*), having been especially studied by Kobert and Ford and Abel.

Relation to Infection and Immunity.—Ehrlich and his colleagues conducted a large part of the pioneer investigations in immunity with abrin, ricin, and crotin because they were more stable than bacterial toxins, produced well-defined test-tube reactions of hemolysis and hemagglutination and proved antigenic for animals, enabling the production of immune sera which neutralized the poisons in a manner analogous to the neutralization of diphtheria toxin by antitoxin. These investigations focused attention upon these plant poisons; Ehrlich believed that they possess the same theoretic structure as bacterial exotoxins, namely, the presence of toxophore and haptophore groups. Since specific antitoxins may be prepared for some of them, they are regarded as true toxins similar to the exotoxins of bacteria.

Of course, not all of the poisons to be obtained from plants can be classed as phytotoxins in this meaning of the term; some, like the saponin substances and various alkaloids, do not produce antitoxins when injected into animals. True phytotoxins, similar to bacterial exotoxins, are probably of protein nature and serve as antigens with the production of specific antitoxins when injected into animals subcutaneously or intravenously, or even by feeding.

Action of Phytotoxins.—Just what rôle these plant toxins play in the production of disease is difficult to state. It is highly probable that some microfungi produce sufficient exotoxin to produce necrosis of epithelial cells and an inflammatory reaction.

Whether or not the injurious effects of poison ivy and the pollens of certain plants and grasses are due to preformed toxins or to hypersensitiveness to the proteins of these plants, is difficult to state; all available data indicates the latter, and only certain persons appear to suffer from contact with them. Dunbar¹ claims to have secured toxins from many different pollens for which antitoxins could be prepared by the immunization of animals.

Many of the phytotoxins produce agglutination and hemolysis *in vitro* and *in vivo*. According to Field² the agglutinating function of ricin is separate and distinct from the toxic function.

¹ For full review of this subject see Glegg, Jour. Hygiene, 1904, 4; Lefman, Zt. f. Hygiene 1904, 47, 153; Wolff-Eisner, Deut. med. Wchnschr., 1906, 32, 138.

² Jour. Exper. Med., 1910, 12, 551.

Flexner¹ found that ricin and abrin produce histologic changes in animals similar to those caused by diphtheria toxin. Ricin apparently carries a toxin-destroying endothelial cell with the production of hemorrhages. Bunting² has described severe changes in the bone-marrow.

All of this indicates the close relationship of these toxins to pharmacology. Bacteria which are plants are known to contain exotoxins for which antitoxins can be prepared, and endotoxins for which antitoxins cannot be prepared. The higher plants apparently may contain toxins of a protein nature similar to the bacterial exotoxins and other toxins or poisons similar to the endotoxins, which are frequently glucosids.

Nature of Phytotoxins.—Some, and especially those from which antitoxins may be prepared, resemble proteins and have long been referred to as "toxalbumins." Jacoby was able to secure preparations of ricin and abrin that did not give protein reactions, and he regarded them as large molecular colloids, closely resembling the proteins with which they are associated, but still not giving the usual protein reactions. More recent work by Osborne, Mendel, and Harris,³ however, indicates that these toxins are proteins; at any rate, the toxic properties of ricin was found inseparably connected with the coagulable albumin of castor beans, and destructible by tryptic digestion.

Other so-called phytotoxins have been largely identified as glucosids. Ford⁴ found the hemotoxin of *Amanita phalloides*, the phallin of Kobert,⁵ to be a glucosid, but yet capable of acting as an antigen with the production of an antiserum; the thermostabile *Amanita* toxin, on the other hand, is probably an alkaloid, and gives no reactions for either glucosids or proteins. Other mushroom poisons (*Amanita muscaria* and *Helvella esculenta*) and the poisons of *Rhus toxicodendron* and *Rhus diversiloba* are either glucosids or poisons classed as alkaloids.

TOXINS OF PROTOZOA AND HIGHER ANIMALS (ZOOTOXINS)

While many Protozoa are known to produce disease our knowledge of the mechanisms involved is very incomplete, and largely because of the technical difficulties involved in studies bearing upon the production of toxic substances.

It is probable that pathogenic *Treponemata* (syphilis; yaws) produce exotoxins; clinically, certain symptoms in acute syphilis are suggestive of a *toxemia*. Toxic substances are also produced in amebiasis, malaria, and other protozoan infections, although the exact nature of the "toxins" is unknown.

Whether or not the worms produce "toxins" is not definitely known; in infestations with tapeworms there is evidence of antibody production suggesting the absorption of antigenic substances. It is not improbable that a toxic substance is produced by *Trichinella* and that some of the symptoms in helminthiasis are due to toxic products including those of a hemolytic nature. Whether or not these substances are deserving of the designation "toxins" cannot be stated.

The most important animal toxins (zoötoxins) are those of the toad, spider, snake, scorpion, and bee. The most striking characteristic of these

¹ Jour. Exper. Med., 1897, 2, 197.

² Jour. Exper. Med., 1906, 8, 625.

³ Amer. Jour. Physiol., 1905, 14, 259.

⁴ Jour. Infect. Dis., 1906, 3, 191; *ibid.*, 1906, 4, 434.

⁵ St. Petersburg. Med. Wchn., 1891, 16, 463 and 471.

toxins is that an immunity against them can be established; in this respect they resemble true toxins. All are quite complex in structure and properties, and all are more or less hemotoxic.

Snake Venoms.¹—Medically, these are of particular interest. They were first thoroughly investigated by S. Weir Mitchell (1860) and Mitchell and Reichert (1883), and have aroused considerable attention because of their similarity to bacterial toxins and the aid their study has been in the elucidation of immunologic problems.

Properties of Venom.—In 1883 Mitchell and Reichert described two poisonous proteins, constituents of venom, one of which seemed to be a globulin and the other a proteose or "peptone." Faust² believes that the poisons are not proteins, but glucosids free from nitrogen, and that they belong to the saponin group of hemotoxic agents. It may be that these glucosids are bound to proteins, and can be removed with the globulin in fractional separation, or that they may come down, at least in part, with the albumoses of the venom.

Various enzymes have been found in venoms; *e. g.*, proteases (Flexner and Noguchi) and lipases (Noguchi); the latter probably have a definite relation to many of the effects of venom intoxication, especially hemolysis and fatty degeneration of the tissues.

The poisons, as a rule, produce both local and severe general disturbances, the rapidity of the onset of the symptoms and the prognosis in a given case depending largely on the situation of the bite. Most of these poisons exert their effect primarily upon the nervous and vascular systems, besides exhibiting other toxic properties.

Nature of Venoms.—All snake venoms possess a hemolytic power, and venom hemolysis is one of the most interesting of biologic phenomena. Flexner and Noguchi³ have distinguished and classified the various elements as hemotoxins, hemagglutinins, neurotoxins, leukotoxins, and endotheliotoxins (hemorrhagin). The endotheliolytic action of the toxins is shown in the glomerular capillaries, where it causes hemorrhage and hematuria (Pearce⁴).

Cobra hemotoxin is especially characterized by its power of dissolving the corpuscles of certain species (man, dog, guinea-pig, rabbit) without the presence of serum. The explanation of this interesting phenomenon has excited extensive discussion. It is probable that the hemotoxin is in the nature of an amboceptor (Flexner and Noguchi), which is activated, in the absence of serum, by complementing substances (chiefly lecithin) present in the red cells, and in this manner producing hemolysis of these cells. In syphilis the quantity of red-cell lecithin is probably diminished after the primary stage, so that when using definite dilutions of venom that are known to hemolyze a certain quantity of normal erythrocytes, an absence of hemolysis of the red corpuscles of a given patient would infer a decrease in complementing lecithin in these corpuscles and indicate the presence of syphilis. The technic of this reaction and its value as a diagnostic procedure will be discussed further on under the head of **Venom Hemolysis**.

¹ See Faust: *Die tierischen Gifte*, Braunschweig, 1906; Noguchi: *Carnegie Institution Publications*, 1909, No. 111; Calmette: *Les venins, etc.*, Paris, Masson, 1907; Wells, *Chemical Pathology*, W. B. Saunders Co., 1920.

² *Arch. exp. Path. u. Pharm.*, 1907, 56, 236; 1911, 64, 244.

³ *Jour. Exp. Med.*, 1903, 9, 257; *Univ. of Penna. Med. Bull.*, 1902, 15, 345.

⁴ *Jour. Exp. Med.*, 1909, 11, 532.

ENDOTOXINS

It is well known that many pathogenic bacteria produce such small amounts of exotoxin that their toxicity is not to be explained on this basis; this group includes the staphylococci, streptococci, pneumococci, meningococci, gonococci, typhoid-colon group, and numerous other pathogenic and saprophytic micro-organisms. Pfeiffer originally taught that the toxicity of these bacteria was due to a preformed toxin which he called "endotoxin."

It is probable that microfungi and the pathogenic Protozoa, notably the Spirochetes and Treponemata, contain similar poisons.

Definition.—Endotoxins are preformed toxic substances or toxins retained in the bodies of *microparasites until released by disintegrative processes*.

This definition is essentially that given by Pfeiffer, although many investigators doubt the existence of preformed intracellular toxins, and much new light has been thrown upon the probable mechanism of disintegration of bacteria in the course of which these poisons are produced.

Methods for Obtaining Endotoxins.—Endotoxins are obtained from bacteria by thorough disintegration of their bodies. A variety of methods have been proposed, some of which are objectionable because they permit the formation of protein digestive products which are commonly mistaken for preformed endotoxins. Experimentally endotoxins should be obtained with a minimum of heat and as rapidly as possible; the following methods have been used:

1. By cultivating the micro-organism on solid or in liquid media, *rapidly washing young cultures with cold isotonic saline solution by centrifuging*, and suspending in saline at 37° C. for brief periods of time for total or partial autolysis. This is essentially one of the methods employed by Cole¹ for demonstrating an endotoxin in pneumococci. Cole also produced these endotoxins by suspending pneumococci in dilute solutions of bile salts at 37° C. for ten minutes, or for a half hour on ice.

2. By suspending washed micro-organisms in saline solution or distilled water and alternately freezing and thawing. This is essentially the method employed by Rosenow² for securing an endotoxin from pneumococci.

3. By freezing and grinding, as in the process of securing typhoid endotoxin employed by Rowland and Macfayden,³ or by the grinding of dried bacteria as employed by Howlett.⁴

Autolytic digestion by prolonged cultivation in broth or non-nutrient fluids cannot be recommended as time is thereby permitted for the activity of proteolytic enzymes liberated from dead or devitalized bacterial bodies. True or preformed endotoxins should be so designated only when these toxic substances are secured by *rapid disintegration of washed bacterial cells*, as in the methods employed by Cole; furthermore, the fluids should be thoroughly centrifuged to remove bacterial bodies.

The methods advocated by Friedberger and Vallardi,⁵ Neufeld and Dold,⁶ and others, consisting of treating bacteria with immune serum and complement outside of the body, is objectionable because it permits of the production of protein split products from the protoplasm of the bacterial cells; the same objection holds for the alcoholic potash method of Vaughan and

¹Jour. Exper. Med., 1912, 16, 644.

²Jour. Infect. Dis., 1912, 11, 235.

³Centralb. f. Bakt., orig., 1903, 34, 618.

⁴Proc. Roy. Soc. Med. (Path. Sect.), 1911.

⁵Ztschr. f. Immunitätsf., orig., 1910, 7, 94.

⁶Berl. klin. Wchn., 1911, xlviii, 55.

Wheeler,¹ and the watery soda method of Schmittenhelm and Weichardt. These methods have greatly complicated the subject and are discussed in a succeeding section of this chapter in relation to the rôle of proteins and protein split or digestive products in relation to disease.

Properties and Nature of Endotoxins.—Their chemical nature and structure are unknown because of the impossibility of securing endotoxins in pure form and free from other bacterial substances and products. Koch's old tuberculin has long been regarded as an endotoxin liberated during prolonged cultivation of the tubercle bacillus in broth. It appears to be a polypeptid, giving no biuret reaction, but being destroyed by pepsin and trypsin (Laevenstein and Pick). Pick regards tuberculin as a secretory toxin closely related to the true exotoxins. It is probable that some toxin is actually secreted into the culture medium and that the major portion, which is of a somewhat different nature, is intimately related to the protoplasm of the bacterial cells.

Endotoxins are generally more resistant to heat than exotoxins and do not deteriorate as rapidly upon standing. Rosenow found his pneumococcus endotoxin soluble in ether and destructible by heating at 60° C. for twenty minutes and by weak hydrochloric acid. He has suggested that the toxic substance is probably a base containing amino groups of nitrogen.

Satisfactory antitoxins for endotoxins have not been produced, and this is an important point in differentiating between an exotoxin and an endotoxin of any particular micro-organism. Animals immunized against endotoxin develop substances in their serum that are bactericidal, bacteriotropic, and agglutinative to the bacteria from which the poisons were derived, but the serum itself is not antitoxic for the endotoxins. Therapeutic serums for use against infections caused by the endotoxin class of bacteria are largely bacteriolytic and bacteriotropic in action. The endotoxins of some bacteria, and particularly those of streptococci, seem to repel the leukocytes, or exert a negative chemotactic influence, which may effectually retard or entirely prevent phagocytosis; in this respect they resemble the aggressins of Bail. Immune serums owe a portion, at least, of their therapeutic value to the power they possess of overcoming this influence and facilitating phagocytosis. These serums, however, have not proved of as much value as have the diphtheria and tetanus antitoxins in the treatment of the respective infections mentioned, and have proved a check to the progress of serum therapy.

Action of Endotoxins.—The toxicity of endotoxins has been largely determined by intravenous injection in guinea-pigs and rabbits. Most observers have reported the development of symptoms and lesions resembling acute or delayed anaphylaxis as dyspnea and coughing, partial emphysema and focal hemorrhages in the lungs, stomach, intestines, and epicardium. Gradual wasting with fever and death not infrequently follow during immunization of rabbits, goats, and horses.

Aside from these effects it would appear that endotoxins are capable of arresting the phagocytic activity of leukocytes and other cells; in this regard they appear to be antiopsonic. This phase of the subject has been especially studied by Bail, who believes that these effects are due to separate products of bacterial activity designated as "aggressins." Most investigators, however, regard these "aggressins" as endotoxins or toxic products of protein digestion or disintegration. A further discussion of these substances follows:

¹ Jour. Infect. Dis., 1907, 4, 476.

AGGRESSINS

In an attempt to explain certain observations of Koch to the effect that when a tuberculous animal is injected intraperitoneally with a fresh culture of tubercle bacilli it succumbs quickly to an acute attack of the disease, the resulting exudate being composed almost exclusively of lymphocytes, Bail¹ has advanced the hypothesis that bacteria may secrete *aggressins*, or substances that aim to protect the micro-organism by either neutralizing the action of opsonins or directly repelling the body cells and preventing phagocytosis. Bail found that if he removed a tuberculous exudate, sterilized it, and injected it into healthy animals, it had practically no effect. If tubercle bacilli were injected alone, lesions would develop in the usual number of weeks; but if sterile exudate and tubercle bacilli were injected together, death would follow in about twenty-four hours, indicating that the exudate contained a substance that acutely paralyzed the defensive forces of the animal, and thus greatly increased the virulence of the bacilli. That this effect was not the summation of endotoxins in the exudate plus living micro-organisms was shown by Bail, who found that when large quantities of exudate alone were injected no untoward effects resulted, whereas the injection of a small amount of exudate, plus a sublethal dose of bacteria, would regularly produce acute infection and death. Bail, therefore, concluded that the exudate contained a substance that allowed the bacilli to become more aggressive, and for this reason he called this hypothetic substance "aggressin." He assumes that in a tuberculous animal the tissues are permeated with the aggressin, and that when fluid collects in the body cavities after the injection of tubercle bacilli, this fluid contains large quantities of aggressin. This prevents migration and collection of polynuclear leukocytes, but not of lymphocytes, and hence allows the bacilli to develop rapidly, producing acute symptoms. On the other hand, when tubercle bacilli are injected into the peritoneal cavity of a healthy guinea-pig, polynuclear leukocytes which engulf the bacilli are attracted, thus inhibiting their rapid development, there being no aggressin to prevent phagocytosis.

Similar results were obtained with other micro-organisms. Bail inoculated cholera and typhoid bacilli into the pleural and peritoneal cavities of animals, and an acute local infection occurred. From the exudates so produced he removed the bacteria by centrifugalization, and completed the sterilization with antiseptics or with heat at 44° C. The clear fluid obtained was found to possess but mild toxic properties, and large amounts could be injected into animals of the same species without producing any marked effects; when, however, it was injected into an animal together with a sublethal dose of the particular micro-organism, an acute and fatal infection followed. Similar results were secured with the bacilli of dysentery, chicken cholera, pneumonia, and other diseases. Burgers and Hoschi² have described "aggressins" for dysentery and typhoid bacilli and staphylococci.

Bail's Classification of Bacteria.—Bail found that bacteria differed in their power of forming aggressins; he therefore used this principle in making a division of bacteria into three classes, according to their disease-producing power, as dependent largely upon whether or not the micro-organism can produce an aggressin that is active against the protective forces of the host, particularly against opsonins and leukocytes.

1. *Saprophytes*, or those bacteria that, when injected even in large doses, do not produce any characteristic disease.

¹ Wien. klin. Woch., 1905, 8, 14, 16, and 17; Berl. klin. Woch., 1905, 15; Zeit. f. Hyg., 1905, i, 3; Arch. f. Hyg., 1905, 52, 272, and 411.

² Ztschr. f. Immunitätsf., orig., 1909, 2, 31.

2. *True parasites*, or those bacteria that, when injected even in the smallest amounts, will produce disease and death. These are truly virulent, and the number of bacteria increase so rapidly as to be demonstrable in every drop of blood and in all the organs. Examples of true parasites are the bacilli of anthrax and of chicken cholera, the tubercle bacillus for guinea-pigs, and the bacilli of the group of hemorrhagic septicemia for rabbits.

3. *Half or partial parasites* are those bacteria the infectious nature of which depends upon the number of bacteria injected. The smaller the number, the milder the symptoms, until a dose is reached below which no disturbances are produced. Organisms of this class possess some virulence and toxicity, examples being the *Bacillus typhosus* and the *Spirillum cholerae*.

It is to be remembered, however, that these effects are but relative, and dependent upon the organism, the species of animal, and the mode of infection. For example, the bacillus of anthrax is saprophytic for the frog and hen unless the temperature of these animals is brought to the body temperature of the human; a bacillus of the group of hemorrhagic septicemia of rabbits is saprophytic for human beings, a half parasite for the guinea-pig if injected subcutaneously, and a true parasite for the same animal if injected intraperitoneally.

Nature of Aggressins.—The aggressins in inflammatory exudates are presumably substances capable of paralyzing the protective agencies of the body. Bail regards the aggressins as of the nature of endotoxins liberated from the bacteria as a result of bacteriolysis, and believes that they act by paralyzing the polynuclear leukocytes, thereby preventing phagocytosis. In general, the production of these aggressins goes on more actively the greater the resistance to the bacteria; they are produced in greater quantities during the struggle between the bacteria and the body cells, although they may be produced artificially in the test-tube with large numbers of bacteria and a non-poisonous agent (serum or distilled water) which can disintegrate the cells. In this manner Wassermann and Citron have produced "*artificial aggressins*," which act in the same general manner as the "*natural aggressins*" of Bail.

By many the aggressins are regarded as endotoxins, and while they may possess the nature of endotoxic substances, it is to be remembered that there is no definite relation between the poisonous qualities of the aggressins and their power to increase the virulence of an infection. It is probable, as has been shown by Wassermann and Citron, that pathogenic bacteria contain small amounts of natural aggressin. This aggressin may be regarded as a normal antibody of the bacterium against the defensive forces of the body cells of a host. During infection these aggressins or antibodies are naturally greatly increased, as the bacteria require more and more protection. Being contained to some extent within the bacterial cells, the antibodies are somewhat similar to endotoxins: while endotoxins may be regarded as offensive agents of bacteria, aggressins may be their defensive agents. The belief is in keeping with the hypothesis of Welch¹ and also of Walker,² according to which it may be presumed that bacteria, as living cells, when so placed that they are exposed to the defensive forces of their host, are, under favorable conditions stimulated to produce reciprocal antibodies for their protection, and to generate them in increasing amounts as may be necessary.

Bail regards the aggressins as new substances; as already stated others regard them as simple endotoxins; still others believe them to be free bac-

¹ Brit. Med. Jour., 1902, 2, 1105.

² Jour. of Path., 1902, 8, 34.

terial receptors, and that these receptors may combine with bacteriolytic amboceptors, producing, as it were, a deflection of the amboceptors, so that the bacteria themselves are not attacked, and thus continue to proliferate. The action of aggressins is not dependent upon the toxicity of the endotoxins, for the fluid containing them is devoid of toxic effects; at most, therefore, if they are of the nature of receptors, they possess no toxoporous portion. Gal¹ believes that bacterial filtrates, extracts, and aggressins are only end-products of proteolysis; Pokschischewsky² likewise regards the so-called aggressins as toxic protein substances (toxopeptids). d'Herelle³ has observed that the substance produced by bacteria in resistance to the dissolving action of a diastatic enzyme produced by an ultramicrobe or intestinal bacteriophage, described above, acts like an aggressin by paralyzing the phagocytic activities of leukocytes.

Anti-aggressins may be produced experimentally by gradually immunizing animals with sterile exudates, and this immunity may be transferred passively from one animal to the other by inoculation of its immune serum. These antiaggressins are quite specific, and neutralize the aggressins in an exudate. Numokawa⁴ found the sera of rabbits immunized with "aggressins" largely bacteriotropic in activity; Haslam⁵ has successfully immunized guinea-pigs and calves with "blackleg aggressin" (edematous fluid of calves dying in one to three days after inoculation with a pure culture of *Bacillus chauveaui*), and recommends immunization of calves with this fluid for the prevention of blackleg.

BACTERIAL PROTEINS

While in tetanus and diphtheria, exotoxins are the chief and almost sole pathogenic agents, in practically all other bacterial infections the proteins of the micro-organisms are to be considered in addition to exotoxins and endotoxins, as contributing in an important manner to the production of local inflammation and toxemia. The same is probably also true in infections with microfungi, Protozoa, and other animal parasites.

Nature of Bacterial Proteins.—Vaughan⁶ and his co-workers who have studied bacterial proteins quite extensively, regard bacteria as essentially particulate, specific proteins. They have not been able to demonstrate the presence of cellulose and carbohydrates; fats and waxes that may be present are somewhat secondary and less essential constituents or stored food material. The sum total of the work of these observers would indicate that the greater part of bacteria are made up of true proteins, especially nucleoproteins or glyconucleoproteins, and although they may be simple in structure, they are chemically complex—quite as much so as many of the tissues of the higher plants and animals.

When bacterial cellular substances are split up with mineral acids or alkalis they yield ammonia, mono-amino- and diamino-nitrogen, one or more carbohydrate groups, and humin substances. These protein substances are the same as those obtained by the hydrolysis of vegetable and animal proteins.

By digestion with dilute acids or alkalis, especially the latter, in the form of a 2 per cent. solution of sodium hydroxid in *absolute alcohol*, a sol-

¹ Ztschr. f. Immunitätsf., orig., 1912, 14, 685.

² Ztschr. f. Immunitätsf., orig., 1912, 15, 186.

³ Compt. rend. Soc. d. biol., 1920, 83, 97; *ibid.*, 1921, 84, 339, 538.

⁴ Ztschr. f. Immunitätsf., orig., 1909, 3, 172.

⁵ Jour. Immunology, 1920, 5, 539.

⁶ Protein Split Products, Lea & Febiger, 1913.

uble split product is obtained that resembles in some respects the protamins, although they do not all give a satisfactory biuret reaction. This product is highly toxic, but shows no specificity in its action, being the same whether derived from pathogenic or from non-pathogenic bacteria, or from egg albumen or other protein substance. All that is definitely known regarding it is that it is toxic, protein in nature, but simpler in structure than the complex proteins of the bacterial cells themselves.

Massive cultures of colon, typhoid, pneumonia, and diphtheria micro-organisms are grown in special large tanks containing agar; anthrax is grown in Roux flasks, and tubercle bacilli in glycerin beef-tea cultures. After removal of the growths the bacterial cellular substances are washed once or twice with sterile salt solution by decantation, and then repeatedly washed with alcohol, beginning with 50 per cent. and increasing the strength to 95 per cent. The substance is then placed in large Soxhlet flasks and extracted first for one or two days with absolute alcohol, and then for three or four days with ether. These extractions should be thorough in order to remove all traces of fats and waxes.

After extraction the cellular substance is ground, first in porcelain, then in agate mortars, and passed through the finest meshed sieves to remove bits of agar. The person grinding the cellular substance should wear a mask in order to protect himself against poisoning. Vaughan reports that, despite this precaution, several workers have been acutely poisoned, especially with the typhoid bacillus. Of course, there is no danger of infection, as the bacteria are killed during the treatment. If the finely ground cellular substance, in the form of an impalpable powder, is kept in wide-mouthed bottles in a dark place, it will retain its toxicity for years. This powder constitutes the bacterial protein substance, which may be split up by various means. Vaughan found digestion with 2 per cent. caustic soda in absolute alcohol especially satisfactory for extracting the poisonous group from bacterial or any other protein.

A weighed portion of the protein, prepared as above, is placed in a flask, covered with from fifteen to twenty-five times its weight of absolute alcohol in which 2 per cent. of sodium hydroxid has been dissolved. The flask, fitted with a reflux condenser, is heated on the water-bath for one hour, where it is allowed to cool and the insoluble portion collected on a filter. After thorough draining the insoluble part is returned to the flask and the extraction repeated. It has been found that three extractions are necessary in order to split off all the poisonous group. The temperature of these extractions is 78° C., the temperature of boiling absolute alcohol. By this method the protein is split into two portions, one of which is soluble in absolute alcohol and is poisonous, while the other is insoluble in absolute alcohol and is not poisonous (Vaughan).

The insoluble and non-poisonous portion of the cellular proteins shows most of the color reactions for proteins, and contains all the carbohydrate of the unsplit molecule and most of the phosphorus.

Action of Bacterial Proteins.—The effects produced by bacterial proteins are not specific; the protein substance of non-pathogenic bacteria and, indeed, many proteins derived from vegetable and animal sources have equally marked pyogenic properties. All foreign proteins introduced into the circulation of animals are more or less toxic, and the toxic effects of all bacterial proteins are, in general, quite similar and non-specific.

In practically all bacterial bodies after removal of toxins and endotoxins a certain protein residue remains which, when injected into animals, is able to produce various grades of inflammatory reaction leading to tissue necrosis and abscess formation. This substance was first thoroughly studied by Buchner, who named it *bacterial protein*, and regarded it as identical in all bacteria and having no specific toxic action, but characterized in general by its power of exerting a positive chemotactic influence on leukocytes, and thereby favoring the formation of pus. For example, in the development of an ordinary staphylococcus abscess it is probable that the proteins of the cocci, aside from their toxins, aid in producing tissue necrosis and in attracting leukocytes to the infected area. Similarly, an extract of dead tubercle bacilli may produce a tuberculoma or the tissue changes incident to tuberculosis, differing, however, from true tubercle in that they do not

contain living bacilli and consequently are not infectious. When cultures of diphtheria bacilli are filtered and the residue washed, it is found that extracts of the bacterial substances or the bodies of the dead bacilli themselves are quite free from the typical toxin, but the bacterial substances or the proteins isolated from them, when injected into the subcutaneous tissues of animals, are found to produce a strong inflammatory reaction and necrosis of the tissue cells. Bacterial proteins are apparently much more toxic than ordinary proteins, as shown by Schittenhelm and Weichardt,¹ and few produce as much inflammatory reaction.

Bacterial protein substances may be responsible for certain minor anaphylactic reactions, as has been observed occasionally in the administration of ordinary bacterial vaccines. They may bear an important relation to the development of the state of hypersensitiveness of a tuberculous person in the course of a series of tuberculin injections.

In addition to these effects produced by the proteins of bacterial cells, it is highly probable that *split cleavage proteins* are produced through the agency of proteolytic enzymes from devitalized bacteria, leukocytes, and other body cells. Vaughan believes that "ferments" are produced during infection which produce this digestion or cleavage of bacterial protein and that a toxic fraction results similar to that secured with alcoholic potash described above, responsible for the local inflammation, fever, and injury to the nervous system:

Theory of Vaughan.—According to Vaughan and his co-workers, all true proteins contain a common and non-specific poisonous group. This group may be regarded as the central or keystone portion of every protein molecule, with secondary and possibly tertiary subgroups, in which the specific property of different proteins is inherent. When the main or primary group is detached from its subsidiary group, it manifests its poisonous action by the avidity with which it attacks the secondary group of other proteins. These are detached from their normal positions, and consequently deprive the living protein of its power of functioning normally. When proteins are split, the chemical nucleus or non-specific toxic portion is more or less completely set free, and its toxicity varies according to the thoroughness with which the secondary groups have been removed.

The pathogenicity of a bacterium is determined not by its capability of forming a poison, but by the ability it possesses to grow and multiply in the animal body. When, during an infection, a pathogenic micro-organism reaches the deeper tissues, it is not immediately killed by the defensive ferments of the host, but continues to grow and multiply, throwing out a ferment that feeds upon the native proteins of the body cells, tearing them down and building up a specific bacterial protein that may select a certain point of predilection in which it is most prone to accumulate. Thus, the typhoid bacillus accumulates in the adenoid tissue of Peyer's patches on the intestine, the spleen, and the mesenteric glands; the pneumococcus tends to lodge in the lungs; the smallpox virus selects the skin, etc.

The bacterial toxins and viruses, as, e. g., diphtheria toxin and the virus of smallpox, are regarded as ferments of protein nature, capable of attacking native body protein and building up a specific foreign protein. This foreign bacterial protein is formed during the period of incubation of disease when there is no effective resistance on the part of the body cells to its growth and multiplication. During this time the infected person is not ill, so that the foreign protein in itself cannot be toxic, and the body cells are busy preparing and elaborating a new and specific ferment that

¹ Münch. med. Wchn., 1911, 58, 841.

will digest and destroy the foreign protein. When this new ferment becomes active, the first symptoms of disease appear, and the active stage of the disease marks the period over which the parenteral digestion of the foreign protein extends. These specific ferments split up the foreign protein and liberate the toxic portion or the protein poison; this poison is not a toxin and is not specific, but occurs commonly in all proteins.

The characteristic symptoms and lesions caused by the various infectious processes are determined largely by the location of the foreign protein. The poison elaborated is the same in all infectious diseases, and it is the location of the infection, rather than the exact nature of the infecting agent, which gives rise to the more or less characteristic symptoms and lesions of the several infectious diseases.

Death may be produced by the too rapid breaking-up of the foreign protein, and the consequent liberation of a fatal dose of the protein poison; or it may result from a lesion induced by the products of this disruption, such as perforation of the intestine and hemorrhage in typhoid fever, or it may follow from chronic intoxication and consequent exhaustion. If recovery takes place, the individual enjoys an immunity of variable duration, owing to the presence of specific ferments capable of destroying the particular substrata if infection should occur.

It is this power of body cells, when permeated by a foreign protein, to elaborate a specific antiferment by which the protein is destroyed that, in the opinion of Vaughan, forms the basis of a correct understanding of infection and immunity.

Friedberger's Theory.—A similar theory has been advanced by Friedberger and his colleagues¹ to explain the production of toxic substances from bacteria, which they believe are similar to endotoxins and responsible for anaphylaxis. According to these investigators bacteria are broken down by means of bacteriolytic amboceptors and complement with the production of a poison called "anaphylatoxin," held responsible for the general reaction of local inflammation, fever, injury to the central nervous system, etc. All bacteria are supposed to furnish the matrix for such a poison; only the antibodies are regarded as specific. Whenever antibody, its antigen and complement meet cleavage occurs and the poison is produced: as proteolysis continues the split products become non-toxic. This theory is essentially similar to that previously described by Vaughan; Friedberger regards the digestive processes due to antibodies and complement, while Vaughan calls the same substances "ferments."

Similar views are held by Thiele and Embleton,² who believe that endotoxins are not preformed, but produced in the body by protein cleavage processes by complement and antibodies or autolytic ferments.

These investigations by Vaughan, Friedberger, Thiele, and Embleton resulted in the construction of a very simple explanation for the mechanism of the production of diseases by bacteria. Further investigations, however, have considerably weakened their original position and attractiveness. For example, Keysser and Wassermann,³ Bordet,⁴ Nathan,⁵ Jobling and Peterson,⁶ and others have shown that the protein poison of Vaughan, corresponding to the "anaphylotoxins" of Friedberger and the "endotoxins"

¹ Ztschr. f. Immunitätsf., orig., 1910, 6, 179, 299; *ibid.*, 1910, 7, 94, 665, 748.

² Ztschr. f. Immunitätsf., orig., 1913, 19, 643, 666.

³ Ztschr. f. Hyg., 1911, 68, 535.

⁴ Compt. rend. d. l. Soc. d. Biol., 1913, 74, 1213.

⁵ Ztschr. f. Immunitätsf., orig., 1913, 74, 225.

⁶ Jour. Exper. Med., 1914, 19, 485.

of Thiele and Embleton, may be produced *in vitro* without the presence of bacterial protein by substituting such substances as kaolin, agar, and chloroform. From these investigations it would appear that the protein poison is not derived from cleavage of the bacteria, but from the blood constituents. Investigations of Novy and DeKruiff¹ have confirmed this view and have shown that the important factor in the agar experiments is the physical state of the agar and that ferment action is probably not at all involved.

As shown by Moldovan,² Doerr,³ Slatineau and Ciuca,⁴ and others, and confirmed by DeKruiff, even blood and serum can be rendered toxic in a manner similar to the toxicity of blood treated with agar and peptone, by withdrawal and transfusion in the preclot period or after defibrination, poison production, and fibrin formation occurring in the preclot stage.

These studies have reduced the importance of bacterial protein as a source of toxic substances; the specific "ferments" mentioned by Vaughan have not been satisfactorily demonstrated and there is no conclusive evidence that bacterial proteins are digested by amboceptors and complement as described by Friedberger, Thiele, and Embleton. On the contrary, the researches mentioned above indicate a different rôle for bacterial protein, namely, that toxic substances are derived from the blood constituents, the changes being brought about by physical or chemical changes in which bacterial protein may play a rôle which DeKruiff has spoken of as analogous to a catalyst, but otherwise inert.

Toxicity of Digested Bacterial Protein; Autolysis.—However, it is highly probable that during infection the protein of dead bacteria may undergo a process of digestion by means of their own proteolytic enzymes (autolysis), or by enzymes derived from leukocytes, fixed tissue cells, and plasma. It is also probable that digestion of bacteria and other microparasites may release preformed endotoxins. This subject is presented in greater detail in the chapter on Ferments and Antiferments.

Unquestionably these cleavage bacterial proteins are toxic, reducing the coagulation time of the blood, producing fever, leukocytosis, and other symptoms described by Vaughan as "protein fever"; the amount produced from bacteria, however, must be small and constitutes an objection to accepting them as the sole or, at least, very important factors in the production of toxemia as maintained by Vaughan, Friedberger, Thiele, and Embleton.

Briefly summarizing the important relation of bacterial protein to infection and toxemia it may be stated: (1) Whole bacteria and particularly their nucleo proteins are apparently pyogenic; (2) digestion of dead bacterial protein by proteolytic enzymes from bacteria, leukocytes, fixed tissue cells, and plasma results in the production of small amounts of toxic cleavage products, and (3) bacteria or their protein constituents may bring about certain physical or chemical changes resulting in the digestion of blood constituents and the production of a protein poison capable of exciting local inflammation and general toxemia.

It is highly probable that similar changes occur in infections with micro-fungi and Protozoa; all of the investigations referred to above were conducted with bacteria.

¹ Jour. Infect. Dis., 1917, 20, 449.

² Deut. med. Wchnschr., 1910, 36, 2422.

³ Wien. klin. Wchnschr., 1912, 25, 331, 339.

⁴ Compt. rend. d. l. Soc. d. Biol., 1913, 74, 631.

TOXIC EXUDATES AND PTOMAINS

In addition to the effects produced by bacterial proteins, and especially nucleoprotein, in relation to the local inflammatory reactions and toxemia of bacterial infections as discussed in the preceding paragraphs, it is highly probable that proteolytic enzymes from bacteria, leukocytes, fixed tissue cells, and plasma become active in inflammatory exudates under certain conditions, and produce toxic protein cleavage products by digestion of the dead or devitalized constituents of inflammatory exudates and fixed tissue cells.

It is hardly necessary for me to review here the numerous studies that have been made with the enzymes of bacteria, leukocytes, and fixed body cells¹; proteases have been found in all which, under certain circumstances, become active when the restraining activity of antienzymes is checked. Substrates for these enzymes are furnished by dead bacteria as previously discussed, and more importantly, by dead constituents of inflammatory exudates including dead or devitalized fixed body cells of the part infected. Proteoses and peptone appear in the early stages to be followed by leucin, tyrosin, tryptophan, phenols, skatole, indole, aromatic oxy-acids, mercaptan, etc.

These protein cleavage products are known to be extremely toxic for experimental animals and it is reasonable to believe that in certain infections some may be absorbed and contribute important elements to the production of toxemia. This would appear to be especially true in extensive pyogenic infections with large collections of pus as in carbuncle, empyema, suppurative meningitis, burns, extensive suppurating wounds, etc. Furthermore, these toxic substances may actually aid in the production of local inflammatory changes although, fortunately, they also appear to possess bactericidal properties and may aid in the sterilization of pus and localized infections in general, thereby aiding in breaking up a vicious cycle of events.

Ptomains.—It was at one time believed that the symptoms of many diseases were due to the absorption of soluble basic nitrogenous substances produced by bacterial action upon various albumins, these toxic, alkaloid-like substances being known as *ptomains*. It was soon found, however, that the ptomains produced by pathogenic bacteria were insufficient of themselves to cause the symptoms and lesions characteristic of the respective micro-organisms; that they were in general less toxic than the cultures themselves; that the majority of ptomains are not very poisonous; and that they are not specific, since equally potent ptomains are produced by non-pathogenic bacteria. This lack of specificity is in sharp contrast to the toxins. No matter upon what medium a true toxin producer is grown, the toxin is qualitatively the same, whereas the nature and toxicity of ptomains depend upon the micro-organisms, the culture-medium used, the duration of growth, and the quantity of oxygen furnished. The same micro-organism, when grown on different media or under different conditions, may produce totally different ptomains.

Ptomains may, however, produce disease, and even death, when they are ingested with food that has undergone bacterial decomposition. In most instances of meat poisoning, however, which are frequently ascribed to the presence of ptomains, a specific micro-organism, the *Bacillus botulinus*, or a member of the *B. enteritidis* group of Gärtner, is usually responsible.

¹ For an excellent review see Wells: Chemical Pathology, W. B. Saunders Co., 4th ed., 1920, 48-127.

The commonest sources of ptomain poisoning are improperly preserved meats, fish, sausages, cheese, ice-cream, and milk. This subject received full consideration in Vaughan and Novy's "Cellular Toxins."

A number of ptomains are known, and of some the exact chemical constitution has been established. Brieger has separated one from decomposing flesh and cholera cultures, called cadaverin, which Ladenburg has shown to be pentamethylendiamin, and prepared synthetically. Muscarin, isolated by Schmiedberg and Brieger, and tyrotoxin, isolated by Vaughan, are also well known.

In the isolation of bacterial ptomains Breiger's method is generally employed, which consists of acidulating large amounts of culture with hydrochloric acid, boiling, filtering, evaporating the filtrate to a syrupy consistency, dissolving in 96 per cent. alcohol, and precipitating and purifying by means of an alcoholic solution of mercuric chlorid.

Besides occurring in food poisoning, ptomains may be formed as the result of putrefactive processes going on in abscesses, gangrenous areas, and within the gastro-intestinal canal, and enough of these may be absorbed to produce symptoms of intoxication. Under these conditions it is possible for bacteria to produce ptomains that may be absorbed and produce symptoms of intoxication without the bacteria themselves actually gaining entrance to the tissues and, therefore, not constituting, according to our definition, a true infection. Pernicious anemia, chlorosis, and allied conditions have been ascribed to the absorption of such ptomains from the intestinal canal. Obviously it is difficult or impossible to always differentiate between bacterial toxins and bacterial ptomains, or the products of protein decomposition dependent upon bacterial activity, and we can but admit the possibility of the production and absorption of both bacterial toxins and ptomains under certain pathologic conditions. *Most ptomains probably are produced as the result of decomposition of the dead protein medium upon which the bacteria grow, and to a lesser extent by the destruction of the bacterial cells themselves. It is extremely doubtful if ptomains are produced in important quantities by pathogenic bacteria infecting living tissues.*

MECHANICAL ACTION OF BACTERIA

In former years the theory as to the influence of mechanical blocking of vessels with masses of bacteria was regarded with much favor in the etiology of certain infections, particularly anthrax. At the present time this factor has not the same importance, for while it is true that bacterial emboli may occasion harm by blocking important vessels, further researches have shown that it is doubtful if any pathogenic micro-organisms are totally devoid of toxic action, and that their toxins are largely responsible for the tissue changes and symptoms of infections.

It can readily be understood that emboli of micro-organisms may produce metastatic lesions; thus, when staphylococci are injected into the ear vein of a rabbit they produce abscesses in the kidney and heart, and masses of bacteria from an ulcerative endocarditis when carried to different portions of the body will cause abscess formation; but the question in hand, however, deals with the effects of mechanical blocking itself.

Investigations with anthrax bacilli have shown that they are remarkably free from soluble toxins and endotoxins, although the local lesion develops so rapidly and becomes so quickly necrotic as to suggest very strongly the action of some local toxic substance. Cases of human anthrax seldom prove fatal if the lesion is removed and the blood-stream remains

free from the bacilli. Vaughan has shown that anthrax protein possesses toxic qualities, and since the majority of fatal cases of anthrax show enormous numbers of bacilli in the blood-stream and internal organs, it may be that this bacteremia produces an accumulation of toxins which is greatly augmented when the body cells of the host have produced an antiferment that splits up the protein of the bacilli, the combined toxic substances being responsible for the severe symptoms and death.

With protozoan disease, the possibility of serious symptoms following blocking of vessels is far greater and, indeed, the cerebral symptoms of malignant malaria and sleeping sickness may be due in part to the blocking of small, but physiologically important, vessels with masses of plasmodia and *Trypanosoma gambiensi*, together with the absorption of toxic agents and the products of disintegration. Thus Bass, who has successfully cultivated the malarial plasmodium outside of the body, believes that the parasites, after attaining sufficient size, lodge in the capillaries of the body, especially where the blood-current is weakest, and where slight obstruction occurs as the result of the protruding inward of nuclei of the endothelial cells. Here they remain and develop until segmentation takes place. In the meantime other red corpuscles are forced against them, and if the opening in the infected cell is in a favorable location, one or more merozoites pass directly into another cell; if it is not, the merozoites are discharged into the blood-stream and are speedily killed.

PART III

CHAPTER VII

IMMUNITY.—THEORIES OF IMMUNITY

IN the preceding chapter on the mechanism of infection and the production of an infectious disease the statement was frequently made that the microparasites of disease are required to overcome the defensive forces of a host which are ever on guard to protect the organism against parasitic invasion and infection. Certain of these defenses are natural to the host and in a great majority of instances suffice to protect the body against invasion and infection with bacteria, animal parasites, and various inanimate and injurious substances. When, however, these natural defenses are broken down and infection has occurred, the body cells are not usually rendered powerless and completely overcome, for the products of infection serve as a stimulus to the body cells, calling forth renewed cellular activity and the production of various specific defensive weapons, termed antibodies, which maintain an incessant struggle against the invading pathogenic agents in an effort to rid the body of them and to neutralize their products.

Just as microparasites have various offensive weapons, consisting chiefly of their toxins, so, in like manner, the defensive forces of the host are numerous and even more complex. If the toxin of a micro-organism is its chief pathogenic weapon, as, *e. g.*, the soluble and extracellular toxin of the diphtheria or the tetanus bacillus, then the body cells produce an antitoxin as their chief defensive force; if the offensive weapon is largely in the nature of an endotoxin, as, for example, the endotoxins of the typhoid or the cholera bacillus, then a chief antibody is in the nature of a bacteriolysin. In other infections, especially those due to the pyogenic cocci, certain of the body cells, and chiefly the polynuclear leukocytes, are observed in the tissues to have engulfed the invaders bodily (phagocytes) in an endeavor to digest them and neutralize their products. In addition to these chief antibodies, there are others that appear to aid them in their work.

That one attack of many of the infectious diseases may protect the individual against subsequent attacks, or at least render subsequent attacks mild and harmless, is well known. In India and the East for centuries practical advantage has been taken of this observation in the management of smallpox. In order to protect persons against a severe attack of variola they were deliberately brought in contact with a person suffering with a particularly mild form, in the hope that, by inducing a mild attack of short duration, they would thus obtain protection against the severe, disfiguring, and fatal form of the disease.

The practice, however, was not without danger to the individual and to the public at large, as the induced disease would at times become malignant, and constitute a focus of infection for an entire community. When Edward Jenner discovered that inoculation with cowpox virus could not produce smallpox, but would, nevertheless, stimulate the production of

specific antibodies and confer immunity against it, an enormous forward stride was taken that has since proved a priceless boon in helping to rid the world of the dreadful scourge of smallpox.

The object of all these procedures has been to secure a resistance or immunity to smallpox, either by inducing a mild form of the disease or by protecting the individual by means of inoculation with a virus that has been so changed in its passage through a cow as to render it unable to produce smallpox, but yet is capable of stimulating the body cells to produce antibodies that will neutralize the effect of the true virus. This induced resistance to a given infection constitutes *immunity* or *resistance*, and since the body was purposely inoculated and the body cells rendered active in producing the antibodies, this form of resistance is known as *active acquired immunity*.

Many persons recover from an infection that may have been unusually severe, not because the infecting agent became exhausted or died for want of pabulum, but because it had been gradually worsted in the battle with the defensive forces of the host. In many such instances the host is now immune to this infection for a longer or shorter time, because the body cells have been so profoundly impressed that they continue generating defensive weapons or antibodies for some time after the last vestige of the infecting agent has disappeared. Or, on the other hand, the quantity of antibodies may be so great that they may persist for varying periods of time, even for the remainder of life, ever on guard, and ready to overwhelm their specific enemy should it ever again gain access to the tissues.

Here, then, arises the question concerning the mechanism of recovery from an infection, and since this is so intimately concerned with the general subject of resistance to disease, it is considered under the general head of immunity.

Even superficial observation shows that not all persons are equally susceptible to a given disease, and during the course of epidemics it will be seen that some individuals, although freely exposed, escape infection. Certain species of animals may likewise display a uniform resistance to an infection that will readily enough attack another species of the same general family. It has been demonstrated experimentally that a certain pathogenic bacterium will produce a severe infection in one species of animals and not in another. It may frequently be noticed that even though an infection occurs, it is readily overcome by the natural resources of the host, the latter escaping with slight or no symptoms of disease. In other words, certain persons and animals apparently possess a *natural resistance* or *immunity* to disease, which may be general, non-specific, or due to specific antibodies, this type of immunity being frequently relative and seldom absolute.

Definition.—*Immunity, therefore, in a broad sense, is the effective resistance of the organism against any deleterious influence; in the usual and more restricted meaning the term is applied to resistance against infection with vegetable and animal parasites and their products, which are pathogenic for other animals of the same or of different species.*

It should be remembered that immunity means not only the ability to resist an infection or successful invasion of the tissues by microparasites, but also the continual resistance offered as long as the infection lasts; that is, immunity implies not only resistance to the onset of infection, but also to the course and progress of the resulting infectious disease. The *science of immunity* has, therefore, for its object the study of the mechanism of resistance to and recovery from an infection.

HISTORIC

The development of the science of immunity forms one of the most interesting chapters in the history of medicine. Even in ancient history we can trace the conception of our modern ideas on immunization.

Hippocrates taught that the factor that causes a disease is also capable of curing it—practically the same theory as the more modern homeopathic doctrine of "*similia similibus curantur*." Pliny the Elder recommended the livers of mad dogs as a cure for hydrophobia, thus coming very near to the basis of the Pasteur discovery. As was pointed out by Elizabeth Fraser, the same idea is expounded in the mythologic tale of Telephus, who cured his wound by applying rust from the sword which inflicted it, and in the story of Mithridates, King of Pontus (B. C. 120), who immunized himself against poisons by drinking the blood of ducks that had been treated with the corresponding toxic substances.

Immunization against various venoms has been practised by many of the savage tribes of Africa since earliest times. Mention has previously been made of the method of preventive inoculation against smallpox practised in Asia and other Oriental countries for several centuries by exposing the subjects to mild cases of the disease.

A very definite step in progress must ever be associated with the name of Edward Jenner, who first demonstrated experimentally, and in a scientific manner, that cowpox conveyed to man protected him against smallpox. Jenner was not the first person to make this observation, as many of the Gloucestershire farmers knew that cowpox protected them against smallpox; nor was he the first deliberately to inoculate persons with cowpox virus, as this method had been practised sporadically before his time. Jenner was, however, the first medical man to give the matter serious thought and consideration, and to test the method as thoroughly and scientifically as it was possible to do at that period. Thus, he inoculated with smallpox virus those whom he had previously vaccinated with cowpox virus, and found them immune to smallpox. These experiments were courageously repeated, until a great truth was established, which has resulted in almost completely eradicating the disease from those countries or communities where vaccination is thoroughly carried out. As was to be expected, Jenner met with considerable opposition, and this is readily understood when it is remembered that even today—one hundred and eighteen years later—there are those who refuse to accept, or are unable to realize, the great benefits of this pioneer work. Jenner could not explain his results; he maintained that he was dealing with a modified form of smallpox. We of today have no better means of establishing the truth of the efficiency of cowpox vaccination, nor have we improved any on his method. The specific germ of smallpox is still undiscovered, and we must agree with Jenner that cowpox is probably a modified form of smallpox and practically harmless, the virus of cowpox being the virus of smallpox modified, attenuated, and rendered practically innocuous by passage through a lower animal.

Nothing further of importance was accomplished during the following eighty years, until the next and even greater epoch ushered in the discoveries in bacteriology and the first immunization by Pasteur based on scientific reasoning. The chickens around Paris were being destroyed by a virulent intestinal infection, and Pasteur first isolated the causative micro-organism, a minute bacillus, which he found was capable of producing the disease experimentally in healthy chickens. Quite by accident, so it seemed, he discovered that cultures of this bacillus could by pro-

longed cultivation be attenuated, for when these cultures were inoculated into chickens, the fowls did not die or suffer any ill consequences; further, and what was of the utmost importance, when these same chickens were inoculated with virulent cultures, they were found to be immune to chicken cholera. Here, then, was the key to active immunization in the prevention of disease, and Pasteur possessed the genius to realize the full significance of his discovery.

Armed with this knowledge Pasteur and his assistants, Roux and Chamberland, next studied anthrax, an infectious disease of cattle that was causing a great annual loss to the farmers of France, and the bacillus of which was among the first pathogenic micro-organisms to be discovered. It was found that prolonged cultivation of this bacillus was insufficient to attenuate the cultures, as the spores were highly resistant and retained their pathogenicity under extreme circumstances and over prolonged periods of time.

In 1880 Touissant published a method of attenuating the bacilli by heating the blood of an infected animal to 55° C. for a few minutes; later, Chauveau secured similar results by heating fresh cultures for a few minutes at 80° C. Both methods were uncertain, and neither safe nor practical. After prolonged experimentation Pasteur found that cultivating the bacilli at the relatively high temperature of from 42° to 43° C. resulted in gradual attenuation, and if this cultivation was continued, the cultures were robbed entirely of their disease-producing power. Further, subcultures of these growths when kept at 37° C. did not regain their original virulence, but maintained for generations the grade of attenuation reached in the original culture the result of cultivation for a certain number of days at the higher temperature. In this manner Pasteur was able to control to some extent the degree of attenuation, and by inoculating first a highly attenuated and later a less markedly attenuated culture he could immunize animals against anthrax. This discovery was soon amply verified. The original method is practically the one employed today, and is proving of considerable economic value.

Pasteur's next great experiment was undertaken for the relief of rabies, a condition in which for the first time he came to deal with a disease that not infrequently affects man. His success and the results of his discovery of an effective means of immunization against hydrophobia were even greater than in previous experiments. Here he was dealing with a disease of unknown etiology, the causative agent of which he could not cultivate artificially, but which he sought to attenuate by a new process—that of drying.

Pasteur first established that the virus of rabies is contained within the tissues of the brain and spinal cords of infected animals.

He then invented a method of inoculating animals by making subdural injections of an emulsion of these tissues. By repeated passage of a virus through a number of rabbits a virus of fixed pathogenic power (*virus fixe*) was obtained. By inoculating rabbits with this virus and removing their spinal cords immediately after death and drying these over a desiccating agent at room temperature, he found that he could modify the virulence of the virus at will, depending on the length of the period of drying. By emulsifying small portions of attenuated spinal cord in salt solutions and injecting these he was able gradually to immunize animals against rabies, and finally he applied the treatment successfully to the prevention of rabies in the human being.

Antirabic vaccination is largely responsible for extending our knowl-

edge of the possibility of securing immunization. Pasteur has taught us at least three different methods for modifying a virus in the preparation of a vaccine, and that each disease, being itself a special entity, having its own characteristics, must be dealt with along special lines.

These discoveries were largely empirical, and the explanations of their mechanism are now only of historic interest. It was not until 1883, when Metchnikoff shed light upon the problems of immunity by making a series of remarkable studies on the rôle played by certain of the body cells in overcoming infection, and the part they played in the processes of immunity in general, that the world was given a glimpse into the dark problems of immunity. These observations were soon followed by investigations showing the importance of the body fluids, and since that time a great deal of work has been done upon these subjects. As a consequence, a large amount of data of a wholly new order has accumulated, accompanied by the introduction of a host of new terms expressing diverse views and theories advanced by individual workers. Of the many theories advanced from time to time to explain the phenomenon of immunity, two have claimed the most attention: one ascribes protection and cure to the activity of certain body cells; this is known as the *cellular theory*; and the other attributes these qualities to the body fluids—the *humoral theory*. The chief exponent of the former is Metchnikoff, with his *theory of phagocytosis*, whereas Ehrlich is the father and leader of the latter, with that marvelous invention of human ingenuity, the *side-chain theory*.

OBSELETE THEORIES OF IMMUNITY

The earlier hypotheses advanced by various investigators are now only of historic interest, as in the light of subsequent discoveries and observations they have failed to offer adequate explanations.

Pasteur's own theory and explanation of the mechanism of acquired immunity sought to show that the micro-organisms living in the infected animal used up some substance essential to their existence, so that, for lack of proper nourishment, the micro-organisms were eventually forced to retire, the soil being unfit for further occupation. Pasteur¹ expressed himself as follows on results of experiments with the bacillus of fowl cholera: "The muscle which has been much affected has, even after healing and repair, become in some way incapable of supporting the growth of the micro-organism, as if the latter, by a previous culture, had eliminated from the muscle some principle that life does not bring back and whose absence prevents the development of the small organism. There is no doubt that this explanation, to which the plainest facts at the moment lead us, will become general and applicable to all the virulent disease." This was known as the "*exhaustion theory*."

Chauveau considered it more probable that the micro-organisms, after having lived in the body of an infected animal, produced substances that, accumulating in the blood, had an inhibitory action on the bacteria and were inimical to their further existence. This was known as the "*retention theory*," and in some particulars was just the opposite of the exhaustion theory. Mention may also be made of the theory of Buchner, which ascribed immunity to the property of the animal organism to reinforce local resistance of the organs by means of an inflammatory reaction which protected these tissues against reinfection by the same germ for indefinite periods.

THEORY OF PHAGOCYTOSIS AND CELLULAR RESISTANCE AND IMMUNITY
CHEMOTAXIS

Historic.—As Lord Lister¹ stated in 1896, "If ever there was a romantic chapter in pathology, it has surely been that of the story of phagocytosis." The author of this "story" is Elie Metchnikoff.² His early researches on phagocytosis in the lowly organized forms of life constituted the starting point for an entirely new series of researches on the subject of Immunity, and his treatise on the "Comparative Pathology of Inflammation" must ever remain a most entertaining work and a medical classic.

Several observers before Metchnikoff had considered that leukocytes might assist in bringing about the destruction of microparasites. Panum³ (1874) suggested that the bacilli of putrefaction might, by making their way into the blood-corpuscles and being carried off to the lymph-glands, spleen, etc., thus disappear from the body fluids. Carl Roser (1881) had also observed the ability of certain "contractile cells" to ingest the enemy that enters the animal body. These statements, however, were poorly supported by scientific data, and the subject was not followed in subsequent research. As the result of zoölogic studies, Metchnikoff was led to discover the part played by body cells in the processes of immunity. He observed that when a food particle arrives in the vicinity of a simple unicellular organism as an ameba, the latter, by reason of its "irritability," moves forward and sends out processes of its protoplasm (pseudopodia) that flow around the particle and finally gather it into the interior of the cell. The particle then undergoes a process of intracellular digestion, losing its sharp outline and clear appearance, becoming granular, and disappearing within the protoplasm of its host. Similar studies were made of the processes of nutrition in many unicellular animals, then through actinins, sponges, and similar animals of transparent and simple organization.

Metchnikoff was primarily a biologist, and up to this time was mainly interested in the processes of nutrition in the simple forms of animal life. At this stage, however, he became greatly impressed by Cohnheim's description of the phenomena of inflammation. The diapedesis of leukocytes through the walls of the blood-vessels in an inflammatory reaction impressed him as significant and similar to the movements of the ameba in its process of feeding, and led to comparative studies in inflammation in the lower forms of life, where processes were much simpler to watch and may indicate what occurs in the higher vertebrates.

He found that when daphnias are invaded by the spores of a yeast—the monospora—they may multiply in the body of the host and bring about its destruction. When, however, a few spores gained access, he found that the daphnia's leukocytes approached them, formed a wall around them, and finally brought about their destruction by a process of digestion. He also observed that if rose prickles were stuck into large bipinnaria larvæ of star fish, these were soon enveloped in a mass of ameboïd cells. From this he concluded that, in inflammation, the exudation of leukocytes may be regarded as a reaction against any sort of injury, whether mechanical or due to bacterial invasion.

Metchnikoff traced this defensive reaction against an invading micro-organism from small invertebrates up to man, and showed that instead

¹ Reports, Brit. Assoc. Adv. Sci., London, 1896, p. 26.

² For a complete review of the work of Metchnikoff and his students read Metchnikoff's *Immunity in Infective Diseases*; English translation by Binnie, published by the Macmillan Company of New York, 1905.

³ Virchow's Archiv., 1874, lx, 347.

of bacteria attacking leukocytes and forcing a passage into them, as was then believed, they were indeed pursued and engulfed by the leukocytes. Connecting his various discoveries, he was able to formulate the idea that "the intracellular digestion of unicellular organisms and of many invertebrates had been hereditarily transmitted to the higher animals, and retained in them by the ameboid cells of mesodermic origin. These cells, being capable of ingesting and digesting all kinds of histologic elements, may apply the same power to the destruction of micro-organisms." To a cell and especially to a leukocyte possessing this activity and power he applied the name *phagocyte*, because of its ability to act as a scavenger, gathering up the living and dead material.

The Original Theory of Phagocytosis.—The theory as originally advanced by Metchnikoff regarded the leukocytes and certain other cells as specific fighting cells, able to engulf and consume living as well as dead bacteria and cellular debris. The outcome of any infection would depend upon the success or failure of the phagocytes to overcome the invaders: if they were successful in ingesting all the bacteria, their victory meant recovery; if, on the other hand, they were destroyed by the invaders, the host was overcome by the infection.

Phagocytes may ingest not only living and dead bacteria, but also red corpuscles, cellular debris, inorganic particles, such as coal-dust, and even soluble substances, such as bacterial toxins.

Subsequent discoveries have shown that many other factors are present that considerably modify the workings of so simple a process. Metchnikoff has, therefore, modified his theory from time to time as new discoveries were made, but has always preserved the primary importance of the phagocyte itself.

Before stating the revised theory as it stands today, we will describe the kind of cells that may act as phagocytes, and consider the methods and reasons why these cells assume the functions of phagocytes.

Varieties of Phagocytes.—Not only leukocytes, but other body cells, have been found active in the processes of phagocytosis. Metchnikoff has divided the phagocytes into two great classes:

1. *Microphages*—principally the polymorphonuclear neutrophilic leukocytes (see Fig. 57). The eosinophilic leukocytes are also included in this group, but are of doubtful importance and weak in phagocytic powers. The small lymphocyte may be included in this class, although it is usually considered with the second group.

2. *Macrophages*, principally the large mononuclear leukocytes; ameboid cells of the spleen and lymphatic glands; alveolar cells of the lung; endothelial cells of the serous cavities and lymph-spaces; bone-corpuscles and giant-cells of bone-marrow and embryonic connective-tissue cells (Figs. 55 and 56). As shown experimentally by Jones and Rous the phagocytic powers of fibroblasts appear to be very feeble.¹ These investigators have shown that phagocytosis of blood-pigment, bacteria, etc., which takes place in granulation tissue is probably carried on wholly by endothelial and wandering cells.

The most important are the leukocytes, especially the polymorphonuclear leukocytes, and the large lymphocytes of the blood. All the leukocytes, however, have phagocytic powers, as is well seen in opsonic determinations. Eosinophils are seldom known to ingest bacteria, but in infections with animal parasites, or after the injection of extracts of animal parasites, both a local and a general increase in eosinophilous forms may be observed.

¹ Jour. Exper. Med., 1917, 25, 189.

Small lymphocytes are much less active than the large, presumably because they contain less of the mobile cytoplasm, and consist chiefly of the structurally fixed nuclear substance, and while they take up but a small number of bacteria, they may be observed to contain various other cells, such as red corpuscles and cellular débris.

Besides the leukocytes, some of the tissue cells which are free or have the power of becoming so are actively phagocytic. Endothelial cells of the lymph-spaces and serous cavities are especially active, not only in the phagocytosis of other cells and cellular débris, but also of various bacteria. The experiments of Keyes¹ have been particularly significant in this connection. He has shown that the high immunity of the pigeon to virulent pneumococci injected into the blood-stream is apparently due, in part at least, to their rapid phagocytosis by the hemophagic cells in the liver and spleen. A similar destruction of pneumococci in the liver and spleen has been shown to occur by Berry and Melick,² after intraperitoneal injection.

In exceptional instances epithelial cells may act as phagocytes. In the presence of an irritant these cells may become detached and act as phagocytes; this is exemplified in the case of chronic passive congestion of the lungs, in which the alveolar cells of the lung ingest the hemosiderin formed and deposited (heart failure cells).

Relation of the Cell Types to Infection and Phagocytosis.—The kind of cells that take part in phagocytosis is determined to some extent by the nature of the irritant. Thus, in acute pyogenic infections the polymorphonuclear cell is found to be most active (see Fig. 57). It is extremely rare to find these cells containing bacilli in the tissues, although they will take them up readily enough under the artificial conditions of an opsonic determination (see Fig. 70). In chronic bacterial infection, such as tuberculosis and syphilis, and in infections with various fungi, the small lymphocyte and macrophages are the types most concerned.

Experimental evidence regarding lymphocytic activity is quite contradictory. Undoubtedly many of the cells in the lymphocytic accumulations seen in such conditions as tuberculosis and syphilis are not really lymphocytes from the blood, but are newly formed cells of the tissues. There is no direct means of inducing experimentally a local accumulation of lymphocytes similar to that induced by most any irritant, resulting in an outpouring of polymorphonuclear cells. Long-continued intoxication of animals may result in increasing the numbers of lymphocytes, but the local introduction of the toxin leads to an accumulation of polynuclear cells, rather than lymphocytes. Reckzeli³ found that in lymphatic leukemia, in which the lymphocytes greatly exceed the polynuclears, the pus from an acute lesion or the fluid from the vesicles produced by cantharides, contained practically no lymphocytes, but was composed of the usual polynuclear cell forms. Wlassow and Sepp⁴ state that lymphocytes are not capable of ameboid movement or phagocytosis at ordinary body temperature; Wolff,⁵ on the other hand, claims that tetanus and diphtheria toxins produce lymphocytosis in experimental animals, and Zieler⁶ claims that in the skin of rabbits exposed to the Finsen light active migration of lymphocytes takes place during the reaction. General lymphocytosis may be

¹ Jour. Infect. Dis., 1916, 18, 272.

² Jour. Immunology, 1916, 1, 119.

³ Zeit. f. klin. Med., 1903, 50, 51.

⁴ Virchow's Archives, 1904, 176, 185.

⁵ Berl. klin. Woch., 1904, 41, 1273.

⁶ Centralbl. f. Pathol., 1907, 18, 289.

produced experimentally by the injection of pilocarpin and muscarin, but these bear no relation to the vital process of phagocytosis, as they are apparently extruded from the lymphoid organs by contraction of the smooth muscles (Harvey¹).

As previously stated, the eosinophils undergo a marked increase during infections with various animal parasites.

The typical macrophages, such as the endothelial cells of serous cavities (Figs. 55 and 56) and the lymph-spaces, are mostly concerned in the phagocytosis of other cells and inorganic material. Brodie² considers the phagocytosis of leukocytes and red corpuscles by the endothelial cells of the lymph-glands and the spleen as the normal end of these cells. It is a mistake to believe, however, that they do not ingest bacteria, since endothelial cells are extremely active phagocytically for bacteria; for example, Bartlett and Ozaki³ have shown that the fixed cells of the spleen and liver exert an important rôle in the phagocytosis of staphylococci *in vivo*. On the other hand, polymorphonuclear leukocytes may be observed to contain red corpuscles, especially when aided by a suitable opsonin.

Variations in the Phagocytic Activities of Leukocytes.—Studies *in vitro*, and especially in connection with investigations bearing upon the opsonins, have shown that the leukocytes may vary in phagocytic activity in both health and disease.

Tunncliff⁴ found the leukocytes at birth somewhat less active than in the adult to streptococci, pneumococci, and staphylococci. Their activity diminished considerably during the first month of life and does not reach that of the adult leukocytes until about the third year. This observer has also found that the leukocytes in recent exudates as those produced by intrapleural injections of aleuronaut, are more phagocytic for streptococci, pneumococci, and tubercle bacilli than the leukocytes of the blood of the same animal.⁵

In pneumonia, scarlet fever, erysipelas, and other diseases in which there is acute leukocytosis, the phagocytic power of the leukocytes may be greater than normal, as shown by the experiments of Rosenow,⁶ Potter and Krumwiede,⁷ Tunncliff,⁸ Boughton,⁹ and others. In leukocythemia, on the other hand, Ledingham,¹⁰ Bushnell,¹¹ Keutzler,¹² and others found that the polymorphonuclear leukocytes, and especially the abnormal ones, had deficient phagocytic power.

Glynn and Cox¹³ have shown that during immunization of donkeys with anthrax bacilli, the leukocytes acquire enhanced phagocytic activities for these bacilli as compared with the leukocytes from normal donkey controls. It is probable that the differences are due to the presence of specific opsonins within or upon the leukocytes of the immune animals liberated after washing but, nevertheless, a specific difference between normal and immune cells has been demonstrated.

¹ Jour. Physiol., 1906, 35, 115.

² Jour. Anat. and Physiol., 1901, 35, 142.

³ Jour. Med. Res., 1913, 35, 465; *ibid.*, 1917, 37, 139.

⁴ Jour. Infect. Dis., 1910, 698.

⁵ Trans. Chic. Path. Soc., 1911, 8, 208.

⁶ Jour. Infect. Dis., 1906, 3, 683.

⁷ Jour. Infect. Dis., 1907, 4, 601.

⁸ Jour. Infect. Dis., 1911, 8, 302.

⁹ Jour. Infect. Dis., 1910, 7, 111.

¹⁰ Lancet, London, 1906, February 10, 368.

¹¹ Brit. Med. Jour., 1907, 2, 142.

¹² Ztsch. f. klin. Med., 1909, lxxvii, 131.

¹³ Jour. Path. and Bacteriol., 1911, 16, 535.

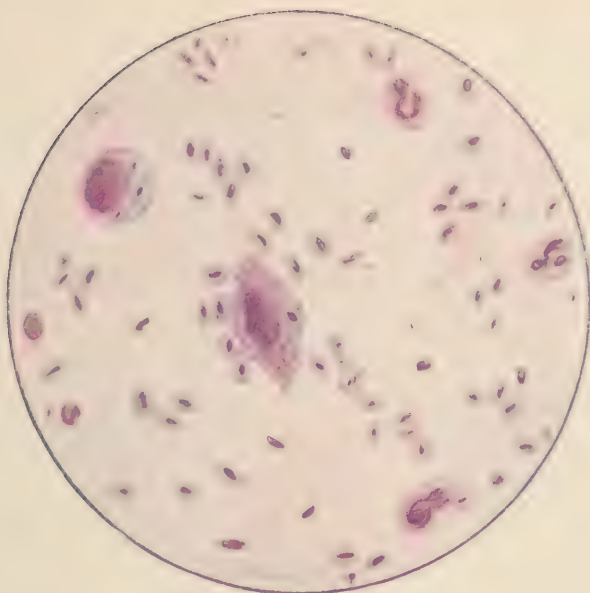


FIG. 55.—PHAGOCYTOSIS—MACROPHAGES.

A smear of peritoneal exudate from a guinea-pig twenty-four hours after injection with 3 c.c. of a 5 per cent. suspension of pigeon's blood. Note that the corpuscles (nucleated) are being ingested by the large endothelial cells of the peritoneum.

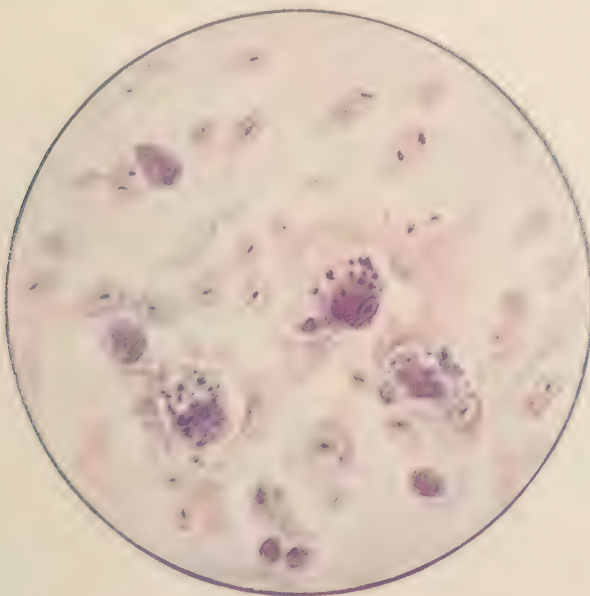


FIG. 56.—PHAGOCYTOSIS—MACROPHAGES.

A smear of peritoneal exudate from the same guinea-pig forty-eight hours after injection with pigeon's blood. Note the large numbers of corpuscles ingested by the endothelial cells. In most instances the corpuscles have undergone digestion, the nuclei being more resistant. These nuclei are shrunken, and in some instances are broken up. The extracellular red blood-corpuscles are swollen and stain lightly.

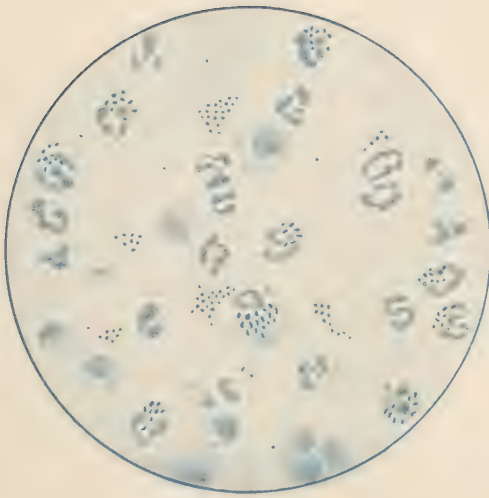


FIG. 57.—POSITIVE CHEMOTAXIS. PHAGOCYTOSIS OF STAPHYLOCOCCI

In certain chronic infections, notably chronic pneumococcus endocarditis and chronic erysipelas, the phagocytic activity of the leukocytes may be either above or below normal. Hektoen¹ has concluded that the plasma independent of its opsonic function may directly influence not only the phagocytic activity of leukocytes, but intraleukocytic digestion as well, the mechanism and significance of these changes being obscure.

These changes in the phagocytic activity of leukocytes renders untenable the principle of Wright that in studying the opsonins the leukocytes may be taken as a constant; in acute as well as in some chronic infections a real grasp of phagocytic activity is obtainable only by the determination of the combined phagocytic powers of the leukocytes and serum of a given patient, and preferably with the infecting rather than a stock strain of the micro-organism. Further reference to this subject will be made in the chapter on Opsonins, because of the influence of the source of leukocytes upon the results of determining the opsonic index in disease.

CHEMOTAXIS

An important question in the study of the phenomena of phagocytosis is the manner in which the various leukocytes and other body cells are attracted to a focus of infection and brought into contact with the micro-parasites or other foreign substances. It must be assumed that some means of communication must exist between this point and the leukocytes in the circulating blood. Since there is no direct communication by way of the nervous system or other structural route, it would appear that the only mode of communication is through the body fluids. *Chemical agencies, produced either directly by the bacteria or other foreign substance, or indirectly by their action upon cells at the site of residence in the tissues, are regarded as furnishing the attractive forces that are transmitted through the body fluids and exert what has been called by Bordet,² chemotaxis.*

The movement of a cell in response to a chemical stimulus is a phenomenon that is displayed by almost all motile and unicellular organisms, whether animal or vegetable, and by the leukocytes and other unfixed cells of the higher animals. As a rule, chemical stimuli serve to attract cells to the site of infection, thus constituting what is known as *positive chemotaxis*; on the other hand, the stimuli may fail to attract or actually repel the cells, or be so powerful as to paralyze them en route, this constituting *negative chemotaxis*.

Positive Chemotaxis.—That leukocytes reach the site of an infection because of chemical substances produced by bacteria at this point was first clearly demonstrated by Leber³ in 1879. This writer observed that in keratitis leukocytes invaded the avascular cornea from the distant vessels, not in an irregular manner, but direct to the point of infection, where they accumulated. As dead cultures of staphylococci produced a similar although a less pronounced accumulation of leukocytes, Leber sought the chemotactic substance in their bodies and isolated a crystalline, heat-resisting substance—phogodin—which attracted leukocytes in the tissues.

Since these fundamental studies were made many other investigations, with various chemical substances of many different origins, have been undertaken upon leukocytes, amebæ, ciliata, and plasmodial forms, indicating

¹ Jour. Amer. Med. Assoc., 1911, lvii, 1579.

² Ann. de l'Inst. Pasteur, 1896, x, 104.

³ Fortschritte der Med., 1888, 6, 460.

that chemical substances are mainly concerned in exerting either a positive or a negative chemotactic influence.

Experimental evidence tends to show that cells respond to stimuli of various kinds chiefly through the effect of these stimuli upon surface tension: if they decrease the surface tension, the cell goes forward; if they increase the tension, the cell recedes.

The behavior of leukocytes in inflammation may be explained on these purely physical grounds. At the site of cell injury or infection chemical substances are produced that tend to lower the surface tension of leukocytes and thus exert a positive chemotactic influence. These chemical stimuli are transmitted by the body fluids to the nearest capillaries, where they enter through the vessel wall and come in relation with the slowly moving peripheral leukocytes. The leukocytes will be brought into touch by the chemotactic substances most largely on the side from which the substances diffuse; accordingly, the surface tension being least nearest the stomata in the capillary wall, this results in the formation of pseudopodia, and motion in this direction, dragging the nucleus along in an apparently passive manner. Those cells, therefore, containing most of the mobile cytoplasm, such as the polynuclear leukocytes, are chiefly affected in these processes; those containing little cytoplasm and a relatively large and dense nucleus, such as the lymphocytes, are affected primarily to a much less extent.

Once outside of the vessel wall, the leukocytes tend to move toward the focus from which the chemotactic substance comes. If the leukocyte meets a substance that greatly lowers its surface tension, it will flow around the object and inclose it, this constituting phagocytosis. The toxins of the ingested bacteria may kill the cell, or so equalize surface tension that movement ceases. Otherwise the leukocytes tend to move forward until checked by any one of several influences, as pointed out by Wells: (1) Until the chemotactic substance has been used up or removed, or from any of the causes that terminate inflammation; (2) the leukocytes may reach a point where the chemical stimulant is so generally diffused that surface tension is decreased equally in all directions and motility stops; (3) the leukocytes may reach a place where toxins or other chemical substances coagulate their cytoplasm or ferments cause their solution; (4) they may be blocked by a dense wall of leukocytes and other cells while being held fixed by the chemical attraction that diffuses through this wall. These factors would explain the formation of the wall of leukocytes about an area of infection. When, for example, the abscess has ruptured or has been incised, with removal of the chemotactic substances, there may be less chemotactic substances in the center of the inflamed area than there is further out; hence, the leukocytes will move away from the center toward the periphery, following the chemotactic substances back into the blood-vessel and lymph-stream. This would explain the dispersion of living leukocytes at the close of an inflammatory process.

General leukocytosis can be explained equally well by assuming that the chemotactic substances from the area of inflammation, reaching the blood-stream, pass through the bone-marrow, lowering surface tension and attracting leukocytes into the blood-stream as long as it contains more chemotactic substances than the marrow.

The exact chemical nature of chemotactic substances is unknown. *In bacterial infection the toxins, and especially the protein of dead micro-organisms, are regarded as mainly responsible for the occurrence of positive chemotaxis.* Chemotaxis and phagocytosis of chemically inert particles, such as coal-dust,

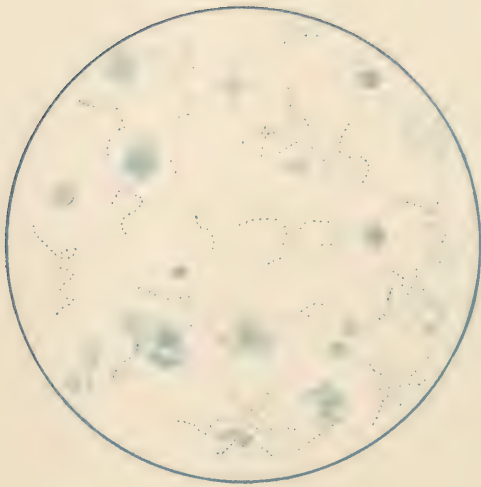


FIG. 58.—NEGATIVE CHEMOTAXIS.

A smear of exudate from the peritoneal cavity of a guinea-pig twenty-four hours after injection with virulent streptococci. The exudate was thin, serous, and tinged with hemoglobin. Note the large numbers of streptococci and relatively few leukocytes.

stone-dust, and pigments, are more difficult to explain on this physical basis of alteration in surface tension. It is probable that the death of tissue cells, brought about by these materials, may produce the chemical stimulant responsible for a mild but definite chemotactic influence. Although the movement of amebæ and similar higher animals cannot be fully explained on this physical basis, the surface tension theory best explains leukocytic movement. Although the ameba may possess some special property that endows it with the power of selecting and engulfing a food particle, it would appear to be entirely unreasonable to assume that a simple, undifferentiated, and naked leukocyte possesses similar powers. The physical theory, therefore, appears to be the most reasonable offered in explanation of the ameboid movements of these simple cells.

Negative Chemotaxis.—In nearly all infections we find that leukocytes are attracted in large numbers into the involved area, *i. e.*, nearly all bacteria give off substances that are positively chemotactic. In certain infections, however, we may find the tissues poor in leukocytes, as exemplified in infections due to the presence of virulent streptococci. This *negative chemotaxis* is more difficult of explanation. Kantlack doubts the existence of really negative chemotactic action upon leukocytes. Verigo¹ also considers that as yet no actual negative chemotactic substances have been satisfactorily demonstrated; certainly no marked example of negative chemotaxis has been shown since methods involving the study of phagocytosis *in vitro* have been devised. It is true that virulent bacteria appear to repel the leukocytes but, as Kantlack has pointed out, these are not necessarily examples of negative chemotaxis, and it is probable that the paucity in numbers of the leukocytes about such an area of inflammation is due to their overstimulation or paralysis and destruction of the powerful ferments that are given off by the bacteria. Thus, Metchnikoff has asserted that leukocytes might, after a time, be attracted toward substances that would kill them. Therefore, while leukocytes will migrate freely toward substances that would kill them, they may be destroyed before they reach the inflammatory area, or, having reached there, are promptly destroyed and pass into solution (Fig. 58).

While it is doubtful, therefore, whether substances are produced by bacteria that actually repel leukocytes, the point has not been definitely settled. If such substances exist, it would appear that they are closely identified with either the *endotoxins* or the *aggressins*, the latter being definite secretory products of bacteria that neutralize opsonins and retard phagocytosis. In many instances it is probable that the same substances that exert a positive chemotaxis are, when concentrated, negatively chemotactic, through overstimulation and paralysis of the leukocytes. With diminution in the numbers or vitality of bacteria and dilution of their chemotactic substances, this inhibiting influence is removed and the leukocytes are attracted to the focus of infection, thus explaining in a way those instances in which positive chemotaxis is observed to follow a primary period of negative chemotaxis. This subject is further discussed below in the section dealing with the influence of bacterial products on phagocytosis.

The Effect of Drugs Upon Phagocytosis.—Cantacuzene,² working in Metchnikoff's laboratory, early showed that phagocytosis of cholera vibrios in the peritoneal cavity of guinea-pigs was retarded during opium narcosis. Since then a great deal of work has been devoted to the influence of chemical substances upon phagocytosis and antibody production in relation to the treatment of disease.

¹ Arch. d. med. Exper., 1901, 13, 585.

Ann. d. Inst. Pasteur, 1898, 12, 288.

Rubin¹ found that alcohol, ether, chloroform, and narcotics in general reduce resistance to infection by affecting the leukocytes or substances derived from them; Hektoen and Ruediger² have shown that various salts are barium chlorid, calcium chlorid, magnesium chlorid, etc., retard phagocytosis in the test-tube presumably by some influence upon opsonins. Hamburger,³ however, has found that very small quantities of calcium salts increase phagocytic power to a considerable extent not only in the test-tube, but also the body. Similarly, numerous other substances—iodoform, benzene, camphor, turpentine, alcohol, chloral, fatty acids, and balsam of Peru—all applied in very small doses, showed a stimulating effect on phagocytosis, but paralyzed when given in greater concentration; inasmuch as these substances are soluble in fats, Hamburger believes that slight quantities of them may dissolve the outer layer of phagocytic cells and thereby increase their plasticity.

In the investigations of Arkin⁴ it appears that the action of chemical substances on phagocytosis varies with their composition and pharmacologic action. Substances which have an inhibitory effect on oxidation, such as chloroform, ether, morphin, potassium cyanid, and alcohol, all depress phagocytosis. On the other hand, substances like iodoxybenzoate which owe their pharmacologic and germicidal action to the presence of physiologically active oxygen and colloidal metals which stimulate oxidative processes, have a stimulating effect on the phagocytosis of streptococci and staphylococci *in vitro*. Substances like caffein and antipyrin which have little if any effect upon oxidation were found to have slight or no influence upon phagocytosis, whereas calcium, magnesium, and mercuric chlorids, quinin, strychnin, and arsenic compounds were believed to stimulate phagocytosis both *in vitro* and *in vivo*. Tunncliffe,⁵ on the other hand, found that dilute solutions of calcium chlorid, sodium salicylate, lactic acid, and magnesium chlorid increased phagocytosis of diphtheria bacilli *in vitro*, but not *in vivo*. Otsubo,⁶ however, found M/8 solutions of certain salts as magnesium sulphate, sodium carbonate, calcium chlorid, and others to diminished phagocytosis of streptococci in mice; higher dilutions did not appear to stimulate phagocytosis either *in vitro* or *in vivo*.

Graham⁷ has found ether depressive for phagocytosis; Bartlett and Ozaki⁸ found phagocytosis of staphylococci *in vivo* reduced in phosphorous poisoning and after chloral anesthesia. Dewey and Nuzum⁹ found the injection of colloidal suspensions of cholesterin in rabbits depressive for phagocytosis and antibody formation. Klecki¹⁰ has reported the stimulating effect of small doses of radium emanations upon phagocytosis of staphylococci and colon bacilli; Irala¹¹ found that ultraviolet rays promoted phagocytosis of staphylococci, but with prolonged exposure phagocytosis was arrested, due to injury of the leukocytes.

¹ Jour. Infect. Dis., 1904, 1, 425.

² Jour. Infect. Dis., 1905, 2, 128.

³ Physikalisch-Chemische Untersuchungen über Phagozyten, etc., Verlag. von J. F. Bergmann, Wiesbaden, 1912.

⁴ Jour. Infect. Dis., 1912, 11, 427; *ibid.*, 1913, 13, 408.

⁵ Jour. Infect. Dis., 1916, 19, 97.

⁶ Jour. Infect. Dis., 1921, 28, 18.

⁷ Jour. Infect. Dis., 1911, 8, 147.

⁸ Jour. Med. Research, 1913, 35, 465; *ibid.*, 1917, 37, 139.

⁹ Jour. Infect. Dis., 1914, 15, 472.

¹⁰ Ztschr. f. Immunitätsf., orig., 1912, 13, 589

¹¹ Ann. d'Igiene, 1920, 30, 28.

THE EFFECT OF BACTERIAL PRODUCTS ON PHAGOCYTOSIS

As recently shown by Wadsworth and Hoppe¹ bacterial products depress the phagocytic activities of leukocytes *in vitro*. Diphtheria toxin causes immediate depression when placed in mixtures of leukocytes and bacteria. This depressor action could not be neutralized by antitoxin, heat, or light, and variations in the constitution of the culture broths, which greatly affected true toxin production, caused no variation in the production of the depressing substance. The depressing action of young culture broths was found to be less marked than that of older cultures. It was also found that digestion with proteolytic enzymes either wholly or partially destroyed the depressing element. The substance could be isolated by absorbing it with leukocytes and then washing it from them with salt solution. After removal of the substance the leukocytes regained their phagocytic activity.

As discussed more completely in the chapter on Ferments and Antiferments the digestion products of enzymes from bacteria and tissue cells may exert a negative chemotactic influence. d'Herelle states that the diastatic ferment produced by his "bacteriophage" and regarded as the active bacteriolytic agent repels phagocytosis.

These and other experiments indicate that phagocytosis *in vivo* may be readily influenced during infection by bacterial products exerting negative chemotaxis.

RESULTS OF PHAGOCYTOSIS

Cytases and Endolysins.—After phagocytosis has been accomplished the fate of the engulfed objects depends upon their nature. In general, they undergo a process of digestion. The ameba, for example, is able to kill and digest engulfed material through an intracellular ferment regarded as a form of trypsin, demonstrated by Mouton² and called *amebadiastase*. According to Metchnikoff, the digestion of erythrocytes and tissue fragments is accomplished through an enzyme of the macrophages called *macrocytase*; that of bacteria or other substances engulfed by microphages by a similar enzyme called *microcytase*.

Following the general law that living protoplasm cannot be digested, we are confronted with the very important question as to whether living bacteria are engulfed by phagocytes or whether they are first destroyed by extracellular agencies before they undergo phagocytosis.

It seems positively established at the present time that leukocytes do take up living bacteria, which may either grow inside the leukocyte or be destroyed by intracellular substances called *endolysins*. On the other hand, leukocytes do not take up extremely virulent bacteria, hence the question arises as to the importance of substances in the body fluids which neutralize the repelling substances of bacteria and facilitate phagocytosis. This subject, which has considerably modified Metchnikoff's views of phagocytosis, will be considered in a succeeding chapter. It will suffice here to state that leukocytes may engulf living bacteria possessing some virulence, for not infrequently an infection may be spread by bacteria transported into deeper tissues by phagocytes, when they resist the germicidal activity of the endolysins, bring about the death of the phagocyte, and are thus liberated into new tissues.

Death of the engulfed bacteria is, therefore, brought about by endolysins³ that are probably different from the digestive enzymes, or cytases

¹ Jour. Immunology, 1921, 6, 399.

² Compt. rend. de l'Acad. Sci. de Paris, 1901, cxxxiii, 244.

³ For general review, see Kling, Ztschr. f. Immunitätsf., 1910, 7, 1.

which bring about the digestion of dead cells. These endolysins are strongly bactericidal and have a complex structure resembling bacteriolysins. According to Weil¹ they are not specific. They are resistant to 65° C. or even higher, do not readily pass through porcelain filters, are precipitated by saturation with ammonium sulphate, and resemble the enzymes in many respects (Manwaring²). It is probable that the endolysins act not only upon bacteria that have been phagocytosed, but also upon free bacteria when liberated through disintegration of the leukocytes. In this manner the endolysins would closely resemble the bacteriolysins and support the contention of Metchnikoff that these important substances contained in the body fluids are derived primarily from the cells which he has classed as phagocytes. According to Schneider³ lymphocytes and macrophages seem to contain little or no endolysin, and these cells are not so active in the phagocytosis of virulent bacteria as are the microphages.

It is possible, however, that in certain instances cells not only fail to kill the microparasites they ingest, but actually protect them from circulating antibodies; apparently the micro-organisms of leprosy, tuberculosis, gonorrhea, and leishmaniosis may live more or less habitually within tissue cells, and, as demonstrated by Roux and Jones,⁴ living phagocytes are able to protect ingested bacteria from the destructive substances in the surrounding fluid, and even from a potent homologous antiserum.

Indigestible substances, if chemically inert, may remain in cells, particularly in fixed tissue cells, for variable periods of time. The leukocytes seem to transfer such particles to other tissues, particularly to the lymph-glands. It is probable that these phagocytes are in turn engulfed by the endothelial cells. Macrophages of the lymph-sinuses or the leukocytes may be destroyed in the glands, and their contents rephagocyted by these cells. In just what manner these insoluble particles reach the gland stroma or perilymphangeal tissues is unknown; it is probable that they are liberated from the lining endothelial cells, and are again seized by the young connective-tissue cells.

THE MECHANISM OF PHAGOCYTOSIS

Aside from the question of whether fixed and wandering cells may engulf virulent microparasites and the influence of substances in the body fluids upon this phase of phagocytosis, we have for consideration the mechanism whereby these cells engulf bacteria and other microparasites, various cells, and inorganic particles. Based upon the direct observations of Metchnikoff and his pupils the phase of engulfing is accomplished by means of pseudopods and the ameboid movement of the phagocytizing cell, whereby the particles become adherent and are finally rolled or passed into the protoplasm of the phagocyte. Recent investigations by Barikine⁵ and Kite and Wherry⁶ would indicate, however, that in so far as leukocytes are concerned these processes are of minor importance, and that phagocytosis depends upon the physical conditions of the surface of the phagocytic cells, a "stickiness" of the leukocytes, whereby bacteria and other particles become adherent, the engulfing being a purely passive process which depends upon protoplasmic streaming within the cells. They believe that

¹ Arch. f. Hyg., 1911, 74, 289.

² Jour. Exper. Med., 1912, 16, 250.

³ Arch. f. Hyg., 1909, 70, 40.

⁴ Jour. Exper. Med., 1916, 23, 601.

⁵ Ztschr. f. Immunitätsf., orig., 1910, 8, 72.

⁶ Jour. Infect. Dis., 1915, 16, 109.

such substances as opsonins act in increasing phagocytosis merely because they increase the "stickiness" of the cells, and that phagocytosis depends essentially upon the relative stickiness of phagocytes and bacteria; increased phagocytosis in the presence of serum and particularly unheated serum is ascribed to an increased stickiness of the leukocytes. Mechanical contact as well as certain variations in chemical reactions may, therefore, result in the production of those changes in contour and protoplasmic streaming responsible for rolling the particle within the protoplasm of the cell.

The researches of Lawson¹ add emphasis to these observations. Contrary to current opinion, she believes that the malarial parasite is not intracellular, but extracellular and possibly pericellular. They become attached to the erythrocytes at "mounds" which the parasites tend to surround with pseudopods.

These researches indicate, therefore, a purely physical base in explanation of phagocytosis tending to render untenable the older conceptions of the mechanism involved.

THE RELATION OF THE BODY FLUIDS TO PHAGOCYTOSIS

Important and far-reaching as were Metchnikoff's researches and conclusions, they were not allowed to pass unchallenged, especially by the adherents of the humoral school, who were able to show the potent influences of the body fluids in the mechanism of recovery from infections where phagocytosis was little in evidence, or, indeed, where phagocytosis was impossible. It was shown that Metchnikoff's original theory was untenable, and that the leukocyte is almost impotent if removed from the influence of the body fluids.

As demonstrated by Denys, Leclef, Flügge, Nuttall, Pfeiffer, and others, bacteria may be killed, *i. e.*, may undergo a process of lysis or disintegration, by means of substances in the blood-serum entirely independent of phagocytosis. Later researches by Wright, Neufeld, and their co-workers demonstrated most clearly that even in those infections in which phagocytosis was observed and known to be of great importance the bacteria are first prepared for phagocytosis by substances in the body fluids, and that without this preliminary preparation of the bacteria phagocytosis was slight and of little consequence.

Metchnikoff corroborated most of these discoveries, and modified his theory from time to time to meet the developments and keep them within the limits of the phagocytic theory. For example, when bacteriolysis was shown by Bordet to be due to two separate substances in the body fluids, which he called *substance sensibilisatrice* and *alexin* (later renamed by Ehrlich amboceptor and complement respectively), Metchnikoff claimed that this phenomenon was extracellular digestion, similar to the intracellular digestion that occurs within the phagocyte, and brought about by ferments secreted and liberated from leukocytes or other cells classed as phagocytes. He regards alexin as a cytase secreted by leukocytes, or liberated upon their disintegration; similarly the substance sensibilisatrice is regarded as a free ferment (*fixateur*), derived principally from leukocytes, and concerned in preparing the bacterial or other cell for the digestive-like action of the cytase.

Aside from these free ferments that are capable of producing extracellular lysis, Metchnikoff has long known that other substances that aid

¹ Jour. Exper. Med., 1915, 21, 584.

phagocytosis itself may be present in the body fluids; he regards these as of the nature of *stimulins*, or substances that stimulate leukocytes to become more actively phagocytic. On the other hand, Leishman, Wright and Douglas, Neufeld and Rimpau, Hektoen, and others have clearly demonstrated that they facilitate phagocytosis not by stimulating the leukocytes, but rather by lowering the resistance of bacteria or in some way rendering them more vulnerable to phagocytosis (*opsonins*, *bacteriotropins*).

Thus the gap between the original cellular theory, which ascribed protection and cure to phagocytosis pure and simple, and the humoral theory (finally summed up by Ehrlich in his side-chain theory), which ascribed the chief and primary rôles to substances in the body fluids, and relegated phagocytes to a position of secondary importance, regarding them only as scavengers that remove dead or disabled micro-organisms, has been filled with discoveries correlating both processes.

The vitality of the leukocyte is to be regarded as important in the consideration of phagocytosis as a means of defense. While the body fluids are acting upon the invaders, the leukocytes themselves are probably undergoing quantitative and qualitative changes. They are increasing in numbers, and as Rosenow¹ has shown, are undergoing more specific changes. Thus, for instance, the leukocytes from a pneumonia patient were found more vigorous against invasion of the pneumococcus than are those from a normal person, regardless of the influence of serum.

When a microparasite is ingested the process has only begun. Unless suitable endolysins are present and the endotoxin is absorbed or otherwise dealt with, and unless suitable digestive enzymes are secreted and the bacterium is dissolved, the process is useless, or indeed, if viable bacteria are transported to other parts of the body, it may be dangerous.

THE REVISED THEORY AND RÔLE OF PHAGOCYTOSIS IN IMMUNITY

As previously stated, Metchnikoff has revised his theory from time to time, as these discoveries were made on the influence of substances in the body fluids, not only upon phagocytosis itself but also upon the processes of immunity in general. He would regard extracellular cytolysis (bacteriolysis, hemolysis, etc.) as due to the same ferments that bring about the destruction and solution of the ingested bacterium or other cell within the phagocyte, and further, these extracellular ferments are derived from the cells that are classed as phagocytes. By this method of reasoning he would preserve the importance of the phagocytic theory.

In local infections phagocytosis is usually well marked, and no doubt plays an important part in resistance to and recovery from these conditions. Recent investigations by Bull² have shown that following agglutination of various bacteria *in vivo* phagocytosis of the micro-organisms frequently follows. In infections due to the various pathogenic micrococci, as staphylococci, pneumococci, and streptococci, phagocytosis appears to be most active and an important means of overcoming the infection. In other infections, as those due to the typhoid bacillus and allied bacilli, it is probable that extracellular substances or antibodies are chiefly operative in affording protection or in overcoming infection, although certain of these, as the agglutinins and opsonins, facilitate phagocytosis.

The question, then, of the relative importance of the cellular and humoral theories of immunity resolves itself to a consideration of the *origin* of the

¹ Jour. Infect. Dis., 1906, 3, 683.

² Jour. Infect. Dis., 1915, 16, 109.

substances in the body fluids so potent in both processes. If they are derived solely from the cells known to act as phagocytes, then the cellular theory of phagocytosis, in its broader meaning, is the one explanation of the processes of immunity as they are now understood. This, however, has never been proved, and it is entirely likely that these substances are products of a general, rather than of a more restricted, cellular activity, so that ultimately all immunologic processes are cellular in origin. For this reason we prefer to speak of the *phagocytic cell in its relation to immunity* when dealing with the relation and activity of microphages and macrophages in a limited sense in the process of phagocytosis.

HUMORAL THEORY OF IMMUNITY

The *humoral theory of immunity*, which would ascribe the power to resist infection to the body fluids, may be said to have had its origin in 1896, when Fodor¹ discovered that the blood of the rabbit will kill anthrax bacilli in the test-tube independent of cells and phagocytosis. Under the inspiration of Flüge,² Nuttall³ confirmed Fodor's results and went further, showing that the bactericidal power of the blood and other fluids is due to a substance of undetermined nature which is destroyed by heating to 55° C. for one hour. Flüge now based a theory of immunity on the presence of bactericidal substances in the body fluids, which was adopted and developed by Bouchard⁴ and his school. Later Buchner⁵ adopted this theory, and sought to explain the bactericidal action of blood-serum as dependent upon a special constituent which he called *alexin* (protective substance).

With the discovery, in 1890, of antitoxins by von Behring and Kitasato the theory received fresh support, and while an effort was made to demonstrate that antitoxins were of paramount importance in acquired immunity, evidence soon accumulated to show that this antitoxic power is operative only in a few diseases, chiefly in diphtheria and tetanus.

Fresh support to the "humoral" as against the "cellular" explanation of immunity was given by Pfeiffer⁶ in 1894, with the discovery that cholera vibrios introduced into the peritoneal cavity of a guinea-pig previously immunized against cholera became transformed into granules, and ultimately passed into complete solution (bacteriolysis), apparently without the aid of cells. Bordet⁷ then showed that this phenomenon was due to two distinct substances—one, the "sensitizing substance," which is specific and exists only in the immune serum, acting only on the bacteria against which the animal was immunized, and the other a non-specific substance, found in the fresh serum of practically all animals, and to which he gave the name "alexin," and which was later renamed by Ehrlich and called "complement."

Of the various theories offered in explanation of these observations, the suggestive, fascinating, though highly hypothetic theory of Ehrlich,⁸ known as the *side-chain theory*, has been most widely accepted and adopted to explain new discoveries as they were made. The theory has, indeed, aided

¹ Deutsch. med. Wchn., 1886, 617; *ibid.*, 1887, 745.

² Zt. f. Hyg., 1888, 4, 208.

³ Zt. f. Hyg., 1888, 4, 353.

⁴ Les microbes pathogenes, Paris, 1892.

⁵ Archiv. f. Hyg., 1890, 10, 84; Centralb. f. Bakteriöl., 1889, 5, 817; *ibid.*, 1889, 6, 1, 561; *ibid.*, 1889, 8, 65.

⁶ Zt. f. Hyg., 1894, 18, 1.

⁷ Ann. d. l'Inst. Pasteur, 1898, 12, 688.

⁸ Klin. Jahrb., 1897, 6, 299; "Croonian Lecture," Proc. Roy. Soc., London, 1900, lxvi, 424.

investigators in making new discoveries. Nevertheless the contention of Bordet, that its too ready acceptance without sufficient convincing proof has retarded investigation, should not be ignored.

The basis of this theory, as originally proposed, bore no relation to the subject of immunity, but was advanced in 1885 to explain the processes of nutrition.

Ehrlich asserts that a cell has two important functions: The first is the special physiologic function, as that of a nerve-cell to conduct; of a gland-cell, to secrete, etc. The second function is that of nutrition, and presides over the processes of waste and repair. Furthermore, each of the molecules composing the complex cell is believed to possess these two functions, *i. e.*, one is concerned with the special function of the molecule, and the other, the more important functional portion, is concerned in the nourishment of the molecule.

The second portion, or that concerned with nutrition, is of more importance in relation to the problems of immunity. Ehrlich conceives this as consisting of a special executive center or main portion ("Leistenkern"), in connection with which there are nutritive side chains, receptors, or haptines ("Leitenketter"), which "seize," or rather enter into chemical combination with, suitable food atoms, which is followed by a sort of digestive or absorptive process, whereby the food material is incorporated in the molecule.

The function of "seizing" molecules of food from the surrounding tissues implies a selective action or chemical affinity between food atoms and the portion of a cell or side arm for which it has a chemical affinity, for we cannot conceive that all atoms that circulate in the blood and lymph are suitable for all cells at all times.

The food molecule in the fluid surrounding the cell is conceived as possessing a special or haptophore portion for union with the side arm of a cell molecule, and when brought into relation with one of the side arms or receptors of the cell molecule, the two are "anchored," or unite, just as a key fits a lock. The second stage involves a process that may be compared to digestion, by which the food material is prepared and absorbed, in whole or in part, into the molecule of protoplasm.

These processes, therefore, are conceived as being chemical rather than physical, and our diagrammatic representations of them have no necessary or actual morphologic basis. One is quite likely to regard the main central portion as the nucleus of a cell, and the side arms as small morphologic projections resembling the prickles of certain epidermal cells. These processes are concerned with each molecule of a cell, the main portion, or "Leistungskern," being conceived as diffusing through the nutritive part of the molecule, and the side-arm receptors, or "Leitenketter," as numerous atoms or groups of atoms, each of which has a chemical affinity for some particular food substance circulating in the body fluids, and necessary for the life of the molecule in question.

Later this theory was amplified by Ehrlich to explain the action of toxins and the production of antitoxins. It assumes that the side arms to a cell molecule are exceedingly numerous, not only because nutritive substances are varied, but because special cells also possess different and special side chains, which anchor pathologic material. When infection occurs, and in addition to toxins the physiologically normal substances are brought to the cells, they likewise find suitable receptors in practically all or certain cell groups, and become anchored, causing more or less damage to the cells.

Having combined with the side arms or receptors of a cell, the toxin may be sufficiently potent to kill the cell, and if a large number of cells are so injured, symptoms of disease present themselves and death of the infected host may follow. On the other hand, although the cell has lost one or more of its side arms, it may not be dead, and it proceeds at once to repair the damage done. According to Weigert's "overproduction theory," nature is lavish in its processes of repair, and the cell not only replaces the lost receptors, but produces them in numbers; the excess receptors, having no space for attachment to the cell, are thrown off into the blood-stream. Each of these cast-off receptors or haptines possesses the same structure as the original receptor. These free receptors, then, are capable of combining chemically with their antigen, neutralizing the antigen, and rendering it innocuous. In diphtheria and tetanus the antigen is largely the soluble toxin of the bacilli, and the antitoxins are these cast-off receptors produced as a result of the stimulating action of the toxins upon the cells. This excess of receptors is made by repeatedly injecting a horse with increasing doses of diphtheria toxin. By injecting this receptor-laden (antitoxin) serum into one suffering from diphtheria the receptors unite with free diphtheria toxin and thus protect the body cells.

For the production of these receptors, or antibodies, as they are now called, it is necessary, as previously stated, that the antigen enter into chemical combination with the cell, so that the usual illustrations showing the theoretic union of antigen and side arm by physical contact alone probably do not correctly portray what actually occurs. As Adami points out, the antigen probably enters into intimate relationship with the cell, and the continued stimulation of its presence is responsible for the production of an excess of receptors, in addition to the overproductive tendencies of nature's repair.

It is also necessary that the antigen possess sufficient toxic power at least to stimulate the cell, for otherwise antibodies may not be produced. Food material, for instance, being physiologic, is assimilated by the cells without stimulating the production of antibodies, as otherwise the food would be attacked by cast-off receptors and rendered useless before it reaches cells, the process ending in starvation and death.

The host in whom certain cells with special receptors for a given poison are present will make use of these, no matter how the pathologic agent is introduced. This affinity is well illustrated in tetanus, where the effects produced are dependent to a large extent upon the selective affinity of the toxin for nerve-cells.

THE THREE ORDERS OF RECEPTORS AND CORRESPONDING ANTIBODIES

First Order: Antitoxin and Simple Antiferments.—The simplest receptor of the cell molecule is composed of a single arm or haptophore, for union with the haptophore portion of a food molecule. As previously stated, a molecule of toxin is conceived as being composed of two portions—one, the haptophore, for union with the side arm or receptor of a cell molecule, and the second, the toxophore, in which its toxic action resides.

The first stage of intoxication of a cell produced by a true toxin consists in the union of the haptophore portion of the toxin molecule to a receptor or side arm of the cell molecule, this receptor being one that fits the toxin molecule "like a key fits a lock." Each molecule of the body cell has innumerable receptors, of which only a certain number are suitable for a particular toxin. The toxin molecule is now anchored to the living cell,

and, as animal experiments with a great number of toxins show, this union is a firm and enduring one (Fig. 59).

So long as the union lasts the side chain involved cannot exercise its normal nutritive physiologic function—the taking up of food-stuffs. Furthermore, the toxophore group of the toxin molecule may now exert an injurious, enzyme-like action on the protoplasm of the cell, with the result that the protoplasm is poisoned. If only a few of the cell receptors are united with toxin molecules, or if the toxin is of low toxicity, the effects on the cell may be slight. If more are joined to the molecule or the toxin is highly poisonous, the whole molecule, and finally the cell itself, may be greatly disturbed, and produce marked symptoms, or the host may be destroyed.

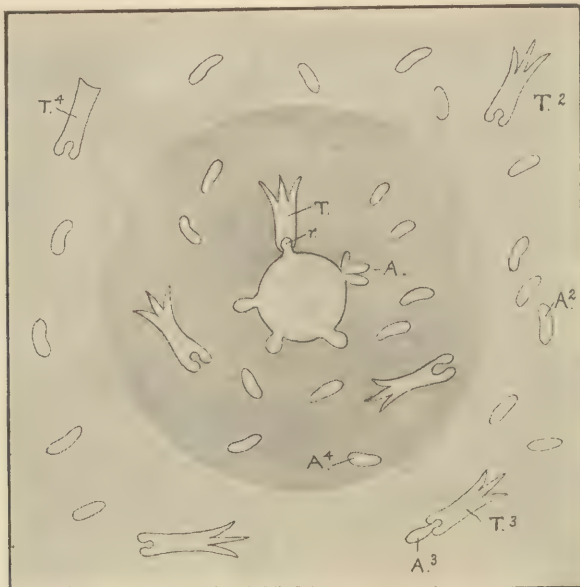


FIG. 59.—THEORETIC FORMATION OF ANTITOXINS.

The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell.

r , A receptor of the molecule (first order); A , overproduction of receptors, which are being cast off; A^2 , a cast-off receptor free in the body fluids—now an antitoxin; A^3 , a molecule of antitoxin combination with a toxin molecule T^3 . A^4 , a cast-off receptor still within the parent cell; T , a toxin molecule in combination with the receptor of a cell molecule; T^2 , a toxin molecule free in the body fluids; T^3 , a toxin molecule in combination with antitoxin; T^4 , a molecule of toxoid (toxophore group lost).

Since the receptors joined to the toxin molecules are incapacitated or destroyed, the damage is repaired by the regeneration of new receptors. According to the reparative principles worked out by Weigert, the repair is not a simple replacement of the defect—the compensation proceeds far beyond the necessary limit; indeed, overcompensation is the rule, and this forms the basis of Ehrlich's theory. If, after repair has taken place, new quantities of toxin are administered at proper intervals and in suitable quantities, the side chains that have been produced by the regenerative process are taken up anew in combination with the toxin, and so again the process of regeneration gives rise to the formation of fresh side chains. "The lasting and ever-increasing regeneration must finally reach a stage at

which such an excess of side chains is produced that, to use a trivial expression, the side chains are present in too great a quantity for the cell to carry, and are, after the manner of a secretion, handed over as a needless ballast to the blood. Regarded in accordance with this conception, the *antitoxins represent nothing more than side chains reproduced in excess during regeneration, and therefore pushed off from the protoplasm and so coming to exist in the free state*" (Ehrlich).

This theory explains the specificity of the antitoxins for a given toxin; thus the latter causes specific chemical stimulation of the cell, which induces the formation of specific side chains—the cast-off receptors—which are capable of uniting with the toxin molecules free in the body fluids and thus neutralizing them; they are, therefore, called antitoxins.

This theory also explains why a minute quantity of toxin is capable of stimulating the production of a large amount of antitoxin, and why the production of antitoxin persists for some time. The toxin molecule must be conceived as entering into the protoplasm of a body molecule and residing there for some time, acting as a stimulus to the cell, with consequent production of antitoxin. Diagrammatic representations of this process would seem to show that a physical union exists between toxin and cell receptors, resulting in the destruction of receptor, which drops off and is replaced by a number of receptors that, for lack of space for attachment to the cell, are thrown off into the blood-stream. In reality, by the act of immunization certain cells of the body become converted into cells that secrete specific antitoxin, and, as shown by Salmonson and Madsen, the administration of pilocarpin, which augments the secretion of most glands, also produces in immunized animals a rapid increase in the antitoxin content of the serum. The formation of antitoxin is constantly going on, and so throughout a long period the antitoxin content of the serum remains nearly constant.

In the production of antitoxin the haptophore group is the essential and important portion of the toxin molecule. Even though the toxophore group is lost—and when this occurs the toxin is called toxoid (Fig. 60)—the haptophore group is capable of uniting with receptors and stimulates the production of antitoxin. In fact, in effecting immunization with powerful toxins it may be necessary, in the first few injections that are given, to convert artificially all or a portion of the toxin into toxoid, so that antitoxins will be produced that will protect the animal against subsequent overdoses of toxin.

The production of antitoxins must, in keeping with this theory, be regarded as a function of the haptophore group of the toxin. It is easy, therefore, to understand why, out of the great number of alkaloids, none is in a position to cause the production of antitoxins, for alkaloids possess no haptophore group that anchors them to the cells of organs. As has been stated, in the formation of antitoxin the haptophore group of the toxin molecule is the essential portion; the toxophore group is much less important, and during immunization the symptoms of illness due to the action of the latter group are not essential to and play no part in the production of antitoxin. It must be said, however, that a toxin molecule with an intact toxophore group is more stimulating than a toxoid in which this group is absent; therefore, in artificially immunizing horses for the production of

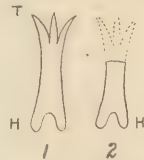


FIG. 60.—THEORETIC STRUCTURE OF A MOLECULE OF TOXIN AND TOXOID.

1, Toxin: H, haptophore group for union with the receptors of cells or antitoxin; T, toxophore group.

2, Toxoid: Same structure as toxin molecule except that the toxophore group is lost.

antitoxin after the first few injections increasing amounts of toxin are administered.

Antibodies of the Second Order (Agglutinins and Precipitins).—As new discoveries were made, Ehrlich amplified his theory of the formation of antibodies, but always upon the original and basic conceptions as just set forth.

We have seen that the simplest molecules of food substances, toxins, and ferments, substances really in solution, are anchored to molecules of cell protoplasm by means of the simple side arms of the latter. When this chemical union has taken place the food or toxin may be assimilated without undergoing any further change. With more complex food substances, however, some preparatory treatment is necessary before they become available for final assimilation. The large molecule may readily enough be anchored to the molecule of the cell, but it probably requires some preparation before it becomes available for the nutrition of the cell.

Accordingly, Ehrlich assumed that the body cells are furnished with another order of side chains or receptors composed of two portions; one part or group for union with the food substance, and called the haptophore group; and the second portion, called the toxophore or zymophore group, in which the special function of the receptor resides.

Similarly, certain pathogenic agents that are more complex than soluble toxins or ferments combine with receptors of this kind. One arm, the haptophore group of the receptor, combines with the haptophore portion of the pathogenic molecule, and then the second or toxophore portion of the receptor exerts some special action upon the attached molecule. Receptors or haptines of this nature are known as receptors of the second order; antibodies of the same structure, produced and cast off into the blood-stream as the result of toxic injury and stimulation of body cells, are known as antibodies of the second order.

Two such antibodies are well known. In one we find that the toxophore group of the antibody causes clumping or agglutination of its antigen, or the agent that caused its production, and hence this antibody is called an *agglutinin*. In typhoid fever, for example, the bacillus or one of its more complex products causes the production of an antibody of this nature, so that when the serum of a typhoid fever patient is mixed with the bacilli, the latter lose their motility and form clumps or agglutinated masses. This phenomenon was first observed by Gruber and Durham, and was applied in a practical way to the diagnosis of typhoid fever by Widal and Grünbaum. The second antibody of this class, the *precipitins*, resemble the agglutinins quite closely (Fig. 61).

Kraus discovered that if a bouillon culture of the typhoid bacillus is filtered through porcelain, and a few drops of serum from a typhoid fever patient or from an animal immunized by injections of typhoid bacilli are added to a small quantity of the bacilli-free filtrate, a faint cloud will appear resembling in some respects that observed at the line of contact between nitric acid and urine that contains a trace of albumin. The toxophore portion of this antibody, therefore, appears to coagulate or *precipitate* soluble substances, and, accordingly, the antibody is known as a *precipitin*. As will be pointed out later, various protein substances, such as blood-serum, milk, egg-albumen, etc., may cause the production of specific precipitins.

Antibodies of the Third Order (Hemolysins, Bacteriolysins, Cytotoxins).—Still more complex molecules of food material require conversion into simpler substances before they may be assimilated by the molecules of the cell. It is essential that they undergo a sort of digestion, and ac-

cordingly Ehrlich has conceived that special side arms or receptors exist for this purpose, these being composed of two grasping portions, or haptophore groups, one for union with the complex food molecule, the second for union with a special, ferment-like substance present in the blood and called *complement*. The receptor, therefore, acts simply as a connecting link or interbody between food molecule and complement, bringing the two into relation with each other when the food molecule is rendered soluble, *i. e.*, undergoes lysis.

With highly organized cell material, such as red blood-corpuscles or bacteria, it is found that receptors of this nature bring about their destruction by lysis by attaching them to a suitable complement. During infections with various bacteria, therefore, we find that numerous anti-

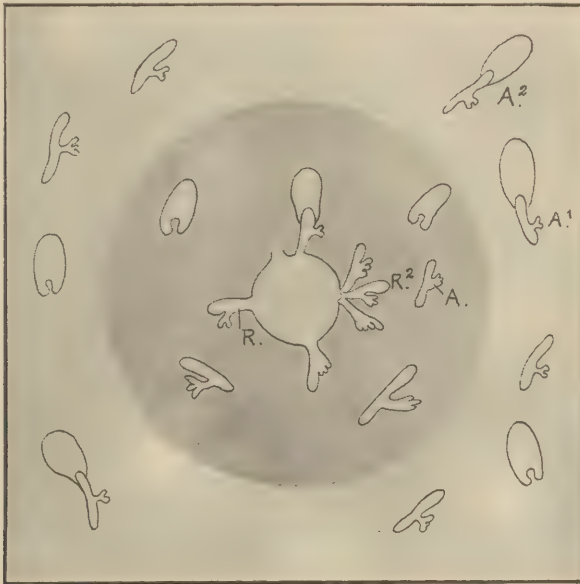


FIG. 61.—FORMATION OF AGGLUTININS AND PRECIPITINS.

The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell.

R, Receptor of the molecule (*second order*); *R²*, overproduction of receptors, which are being cast off; *A*, a cast-off receptor which now constitutes the antibody; *A*, *A²*, agglutinins in combination with the antigen (bacilli).

bodies are produced. If the bacteria produce soluble toxins, specific anti-toxins are produced to counteract the effects of these; other products stimulate the production of agglutinins and precipitins; still other products or the whole cell cause the production of antibodies, which are not in themselves destructive, but which have the specific power of combining with the cell and bringing about its lysis or destruction by bringing it into relation with the ferment-like complement. It is only by means of a special antibody of this nature that a complement may be united with the pathogenic agent, *i. e.*, the complement itself cannot act directly upon the cell, but must be united by means of the antibody.

Ehrlich has termed an antibody of this nature an *amboceptor*, or *inter-body*. In structure, amboceptors are believed to possess two combining

or grasping portions: one, the haptophore or antigenophore group, for union with the cell; the other the complementophile group, for union with a complement (Fig. 62).

The lysins (bacteriolysins, hemolysins, and other cytolsins) are antibodies of this order. If, for example, the erythrocytes of one animal are injected into an animal of a different species, hemolysins will be produced, the hemolysin being a specific hemolytic amboceptor that will unite corpuscles of the animal used in the injection, and only these cells, with a complement, and thus bring about their solution or lysis. If certain bacteria (*e. g.*, the cholera bacillus) are injected into an animal, specific bacteriolysins (bacteriolytic amboceptors) will be produced. Similarly, specific

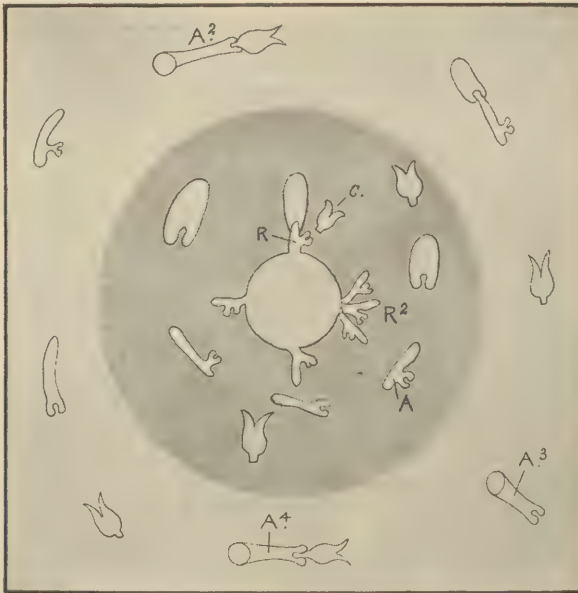


FIG. 62.—FORMATION OF CYTOLYSINS (HEMOLYSINS, BACTERIOLYSINS, CYTOTOXINS).

The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell.

R, Receptor of the molecule (*third order*); *R²*, overproduction of receptors, which are being cast off; *A*, a cast-off receptor which now constitutes the antibody of amboceptor; *C*, molecule of complement free in the body cells and body fluids; *A²*, *A⁴*, amboceptors in combination with molecules of a cell (antigen), and a complement; *A³*, an amboceptor in combination with a molecule of a cell. The cell (antigen) is now said to be sensitized. Lysis does not occur because a complement is not united.

amboceptors are produced during the course of infections with typhoid bacilli, and are largely instrumental in combating and overcoming this infection. It is important to remember, however, that although these amboceptors probably prepare their antigens for lysis, or, in the meaning of Bordet, "sensitize" them, they are not in themselves lytic, final solution of the antigen being accomplished by the ferment-like substance—the complement.

COMPATIBILITY OF THE PHAGOCYTIC AND HUMORAL THEORIES

When we seek to compare the theory of Metchnikoff with that of Ehrlich, we find that they differ only in minor details, the fundamental

principles not being contradictory; they may, rather, be regarded as one set of phenomena viewed from different aspects.

Since its original announcement Metchnikoff has, on different occasions, enlarged upon his theory to meet certain discoveries, made chiefly by adherents of Ehrlich's theory, showing the presence of substances in the blood-serum and other body fluids that are potent in the processes of immunity independent of cells. Metchnikoff claims, however, that these antibodies are derived from the group of cells classified as phagocytes, and thus would preserve the primary importance of his theory. Ehrlich, on the other hand, while not denying that these cells may be a source of their formation, points out that they are not necessarily the sole or supreme source, but may be formed by the general body cells or by special groups of cells possessing a selective affinity for the pathogenic agent.

The theory of Ehrlich is essentially a chemical one, and maintains that the union of food or pathologic material with cells is a chemical union; his views, therefore, possess that degree of definiteness necessary to constitute a plausible chemical theory. The theory of Metchnikoff would explain processes of nutrition and immunity as largely founded on a physical basis, and is, therefore, necessarily more general, being largely biologic and vitalistic.

The two theories differ in two more or less hypothetic points: (1) In the manner by which material enters into relation with cells, and (2) the relative importance of certain cells in the formation of antibodies. Otherwise both are intimately related, in that phagocytosis is unimportant if removed from the influence of antibodies in the body fluids, and these same antibodies, although probably formed according to Ehrlich's theory, are derived in part from Metchnikoff's phagocytes.

Phagocytosis, whether by leukocytes, endothelial cells, or by newly developed connective-tissue cells, is very common, and is obviously a most important factor in the destruction of pathogenic bacteria and in the cure of infectious disease. In virulent infections, however, phagocytosis may not be apparent; the leukocytes are not attracted, and those in the vicinity undergo dissolution. Later in these infections, however, phagocytosis may become apparent, due, according to Metchnikoff, to the "adaptation" of the cells to the products of the invading micro-organism, whereby the weak or negative chemotaxis is converted into an active positive chemotaxis with vigorous digestion. This, however, is not primarily due to increased digestive capacity of the phagocytes, but to an increase of opsonins in the body fluids; these opsonins prepare the bacteria for digestion.

The original phagocytic theory did not explain the destruction of bacteria within the living tissues without the intervention of leukocytes, and, what is even more striking, a similar destruction occurring *in vitro* by serum and other body fluids totally devoid of cells. Bacteriolysis has been shown to be due to two different substances—one, a thermolabile, ferment-like body called "cytase" by Metchnikoff and "complement" by Ehrlich, and the other a more specific thermostabile body, called "fixateur" by Metchnikoff and "amboceptor" by Ehrlich. These substances appear to play an important rôle in certain infections, as, for example, in typhoid fever and cholera, and were studied mainly by the adherents of the side-chain theory. Metchnikoff recognized their existence and significance, but endeavored to preserve the primary importance of the phagocytic theory by claiming that they are products of the group of cells classified as phagocytes. Ehrlich, however, while not denying that these cells may be one source, holds that they are not necessarily the sole source, but that they are products of gen-

eral cellular activity or of special groups of cells that have shown a combining affinity for the antigens.

For example, Metchnikoff holds that there are but two complements—*macrocytase* and *microcytase*—and that these are formed by destruction or solution of macrophages and microphages. Ehrlich maintains that there are many complements, and that these are the excretory products of leukocytes, and probably of other cells as well. Ehrlich teaches also that specific amboceptors or fixateurs may be products of various body cells other than those classified as phagocytes, and Metchnikoff recognizes their existence, but holds that they are formed and discharged solely by the leukocytes or other phagocytic cells. Ehrlich has shown the manner in which complement and amboceptor produce bacteriolysis, and Metchnikoff has amplified his theory to meet these observations, to the extent that destruction of bacteria is recognized as being brought about either intracellularly, by the digestive action of the leukocytes, or extracellularly, by the enzyme-like action of the cytase, or complement, working through the intermediation of the fixateur or amboceptor, and that cells that are potentially phagocytic give origin to these antibodies.

Regarding the structure of toxins and the action of antitoxins the two theories are divergent, and whereas Metchnikoff is inconclusive, Ehrlich presents definite conceptions that are well supported by experimental data. Metchnikoff maintains that it is the cells that absorb the "toxin" that furnish the antitoxin. In other words, the enzymes, as microcytase and macrocytase, exert their action not only upon the more complex molecules of micro-organisms but also upon their simpler toxins, fixing or otherwise altering them until they can finally be destroyed. This explanation would lead us to conclude that the nerve-cells which bind the tetanotoxin are capable of furnishing antitoxin, whereas experimental observations are absolutely opposed to this narrower view. Metchnikoff also maintains that antitoxin acts by stimulating the leukocytes to absorb and destroy toxin, whereas Ehrlich has clearly shown that antitoxin, by combining chemically with the toxin, neutralizes it, a process that may be shown *in vitro* entirely independent of cells.

From what has been said it will be seen that the two theories are not essentially divergent, and that we are unwarranted in clinging to one view to the absolute exclusion of the other. The question rests largely on which of the body cells are most active in forming antibodies, and also on a recognition of the rôle played by phagocytosis in certain infections, such as staphylococcus, streptococcus, and pneumococcus infections. Ehrlich has attempted an explanation of the method by which body cells form antibodies, and the manner in which these antibodies overcome their antigens; he has placed both processes upon a chemical basis, involving no one particular group or class of cells. Metchnikoff, on the other hand, has shown the important rôle played by phagocytosis in many infections, and claims that the antibodies in the circulating fluids are the products of these phagocytes; he places immunity more largely upon a physical basis.

The various phenomena of immunity cannot be ascribed either to the activity of the body cells or to the body fluids alone, to the total exclusion of the other—both are intimately concerned in the various phases of immunity.

It is, moreover, becoming more obvious that too little attention has been paid to the influence of the micro-organism in the phenomena of immunity reactions. It is important to recognize that some bacteria are apparently able to immunize themselves against the combative forces of

their hosts, as is demonstrated by the manner in which streptococci and pneumococci protect themselves with a capsule and resist phagocytosis. Virulent strains and "resistant races" may be evolved in this manner. This has been demonstrated by Ehrlich with regard to the action of various arsenical compounds on protozoa, work that finally culminated in the brilliant discovery of salvarsan. Thus atoxyl may not kill all the trypanosomes in an infected animal, those escaping acquiring a new power of resistance to the poison and become atoxyl-resistant. The production of "resistant races," not only among the protozoa but also in the class of bacteria, complicates enormously the practical problems of immunity.

CHAPTER VIII

ANTIGENS AND ANTIBODIES

ANTIGENS

BRIEFLY defined, *antigens are substances that can cause the formation and appearance of antibodies in the body fluids.*

The number of substances capable of acting as antigens is very large; practically any foreign protein substance introduced parenterally will act as an antigen. For example, living bacteria, fungi and animal parasites and their soluble products act as antigens during infection even when the latter are present in the nature of an intestinal infestation; dead micro-organisms in the form of vaccines may also act as antigens. Any cell or body fluid of one animal may prove an antigen when injected into an animal of a different species. Indeed, the cells of a certain organ may act as an antigen in the same animal when displaced and set free into the circulation for transmission to other parts of the body.

Specific and Non-specific Antigens.—When antigenic substances are injected into heterologous animals the antibodies or protective principles engendered are highly specific for the antigen as a whole or for its constituents. It is now known, however, that the injection of some unrelated and non-specific agent may act as a general stimulant and induce the body cells to throw off small amounts of antibody unrelated to the non-specific agent injected. This has an important bearing upon non-specific "protein therapy" and is discussed more completely in Chapter XXXIX.

Nature of Antigens.—So far as is now known, antigens are colloids, and are usually protein in nature. Every known soluble protein may in some degree act as an antigen, and recent investigations would seem to show, although they do not definitely prove, that toxic glucosids and various lipoids may to some extent act in this same capacity. The protein antigens may be quite varied: thus antibodies are produced not only by the injection of bacteria or their toxins, but also by erythrocytes, serums of different animals, egg-albumen, milk, etc.

Of the cleavage products of proteins, it is certain that none of the amino-acids and simple polypeptids can act as antigens; there is, however, some evidence to show that the proteoses possess antigenic properties. It has been shown by Gay and Robertson¹ that if the antigenic cleavage products of casein are resynthesized by the reverse action of pepsin into a protein resembling paranuclein, this synthetic protein is capable of acting as an antigen. Protamins and globin were found to be non-antigenic, although globin combined with casein formed a compound of antigenic power in that it produced an antibody yielding complement-fixation reactions with globin.

Whether the entire protein molecule, or only groups thereof, determine the characteristics of the antigen and the antibody is not definitely known. Wells and Osborne² have recently submitted evidence showing that a single protein molecule can act as an antigen and produce more than one antibody.

¹ Jour. Biol. Chem., 1912, 12, 233; Jour. Exp. Med., 1912, 16, 479; 1913, 17, 535.

² Jour. Infect. Dis., 1913, 12, 341.

Non-protein Antigens.—Ford¹ was able to immunize rabbits by injecting a toxic glucosid contained in extracts of *Amanita phalloides*, producing an antibody antihemolytic for the hemolysin of *Amanita* when diluted 1 : 1000. Abderhalden and others have found that specific enzymotic substances appear in the blood of animals injected with carbohydrates and fats. Recent developments in immunologic research would indicate, therefore, that a complex toxic glucosid that can be hydrolyzed by enzymes may act as an antigen.

The intimate relationship of lipoids to complement-fixation reactions, especially in syphilis, has naturally led to investigations regarding the possibility of lipoids acting as true antigens. In testing for the Wassermann reaction the use of lipoids in the form of tissue extracts to serve as an antigen does not mean that it is a true antigen; in fact, experimental work indicates quite strongly that these lipoidal substances are incapable of producing antibodies when injected into animals.

Much and others have worked with lipoids secured from a streptothrix, and which is called "nastin," and they assert that this substance may be used in immunizing animals with the production of an antibody yielding complement fixation, with nastin as the antigen. Similar results have been described for the fatty substances from tubercle bacilli ("tuberculo-nastin"). Kleinschmidt² accepts the antigenic nature of nastin in reactions, but was unable to secure antibodies by immunizing rabbits with this substance.

Ritchie and Miller³ could demonstrate no antigenic activity in the lipoids of serum or corpuscles. Thiele⁴ calls attention to the fact that lipoids possess no specificity, and that they cannot act as antigens with the production of antibodies. On the other hand, Meyer⁵ has reported the production of specific complement-fixation antibodies by immunizing rabbits with acetone-insoluble lipoidal substances obtained from various teniæ. He has also found the acetone-insoluble fraction of tubercle bacilli to serve as antigens in complement-fixation reactions with antibodies of the tubercle bacillus, and much more effectively than with the protein residue of the bacilli. Beigel⁶ has observed that after injecting lecithin in rabbits an increase occurs in the lipase content of the blood and tissues, with the presence of complement-fixing antibodies, and Jobling and Bull⁷ have found an increase in serum lipase after immunizing with red corpuscles.

It will be noticed, therefore, that the results of various investigations regarding the true antigenic properties of lipoids are not in accord. It should be emphasized that the complement-fixation reaction does not constitute a reliable index to the study of this problem, as so little is understood of the actual nature of this reaction itself. That lipoids serve a very important purpose in the absorption or fixation of complement *in vitro*, as is so well demonstrated in Wassermann's reaction for syphilis, is undoubtedly true, but this does not indicate that the antibody in the blood-serum of syphilitics is in the nature of a true lipoid antibody, and, indeed, investigation on this subject would seem to indicate that it is not.⁸

¹ Jour. Infect. Dis., 1907, 4, 541.

² Berl. klin. Wchnschr., 1910, 47, 57.

³ Jour. Path. and Bact., 1913, 17, 427.

⁴ Ztschr. f. Immunitätsf., 1913, 16, 160.

⁵ Ztschr. f. Immunitätsf., 1910, 7, 732; 1911, 9, 530; 1912, 16, 355.

⁶ Deut. Arch. f. klin. Med., 1912, 106, 47.

⁷ Jour. Exper. Med., 1912, 16, 483.

⁸ Further discussion on the question of lipoids acting as antigens will be found in Chapter XXVII in a consideration of anaphylactogens.

It will be understood, therefore, that the question of substances other than proteins acting as true antigens must be regarded as an open one, requiring further investigation. The relation of proteins, however, to the production of antibodies has been fully established, and is at present receiving renewed attention through the researches of Vaughan and his co-workers and Abderhalden. As has been stated in a previous chapter, Vaughan regards the protein constituents of bacterial and other calls as the main antigenic principle capable of causing the production of specific proteolytic ferments, which split the new bacterial protein, releasing a toxic product responsible for the symptoms and lesions of the infection. Abderhalden has also demonstrated the presence of proteolytic ferments in the blood-serum after experimental immunization with proteins, and in the serum of pregnant women, due to the antigenic stimulation of syncytial cells, capable of splitting their substrata *in vitro* into amino-acids and other simple cleavage products. These investigations serve to show the intimate relation that proteins bear to the problems of infection and immunity, and demonstrate that antibodies may be largely in the nature of ferments, and that immunologic reactions, both in the living tissues and in the test-tube, are largely in the nature of disintegrative enzymic processes.

Drugs as Antigens.—A large amount of work has been devoted to the subject of formation of protective substances to morphin, cocain, strychnin, and other alkaloids in explanation of tolerance to these drugs. Gioffredi¹ and Hirschlaff² claimed to have produced antimorphin sera of protective value, but Morgenroth,³ in a particularly thorough set of experiments, showed that such sera could not be produced. More recently Pellini and Greenfield⁴ have reached the conclusion that no substance is formed in the blood-serum of a human being who has acquired a high tolerance to morphin, which is capable of conferring any degree of immunity to the toxic action of morphin on an animal into which it is injected. Likewise they have shown that the blood of a tolerant animal does not contain any protective substance against morphin.

ANTIBODIES

The term "antibody" is used to designate the specific bodies produced by the cells of a host in reaction against an antigen, as an infecting microparasite and its products or other foreign protein.

Various kinds of antibodies may be produced by the same antigen and by different antigens. Some neutralize the soluble toxin of the antigen (antitoxin); others agglutinate or precipitate their antigens (agglutinins and precipitins); still others cause complete dissolution of the antigen (hemolysins, bacteriolysins, etc.), and others again may so alter the antigen and lower its resistance as to render it more easily phagocyttable by the body cells (opsonins or bacteriotropins).

Tissues Concerned in the Production of Antibodies.—A large amount of experimental work has been conducted in the study of the problem of where in the body the antibodies are formed that develop in response to immunization. The recent investigations of Hektoen and Curtis,⁵ Gates,⁶ and Launoy,⁷ who studied the effect on antibody production of the removal

¹ Archiv. ital. d. biol., 1897, 28, 402; *ibid.*, 1899, 31, 398.

² Berl. klin. Wchn., 1902, 39, 1149, 1177.

³ Berl. klin. Wchn., 1903, 40, 471.

⁴ Arch. Int. Med., 1920, 26, 279.

⁵ Jour. Infect. Dis., 1915, 17, 409.

⁶ Jour. Exper. Med., 1917, 27, 725.

⁷ Ann. de l'Inst. Pasteur, 1915, 29, 213.

of various organs, and Hektoen¹ and Simonds and Jones,² on the influence of exposure to x-rays, and of Simonds and Jones,³ on the effect of injections of benzol, indicate that the mechanisms concerned for the production of antibodies are quite secure from certain disturbances and are principally located in the leukocytes and blood-forming organs, as the spleen, lymphatic tissues, and bone-marrow.

Carrel and Ingebrigsten⁴ have observed the formation of hemolysin for goat erythrocytes in cultures of guinea-pig's bone-marrow and lymph-gland *in vitro*.

Gowan,⁵ however, does not believe that the spleen, leukocytes, lymphatic apparatus, thyroid, or kidney are principally concerned in antibody production; extirpation experiments did not appear to delay the production of antibody. For various reasons, including the well known function of the liver for removing foreign material from the circulation and its great metabolic activity, Gowan believes that this organ is deserving of more attention from the possibility of playing an important rôle in the formation of antibodies.

In leukemia the presence of very large numbers of leukocytes and hyperactivity of the leukocyto-genic tissues would suggest the possibility of increased antibody production and enhanced resistance to infection. Just the reverse, however, is known to occur. In regard to antibody production both Rotky⁶ and Howell⁷ have found that the leukemic patient produces antibodies poorly or not at all, probably due to excessive proliferative changes in the hematopoietic tissues.

Of further interest in this connection is the question of *local production* of antibodies, that is, by the tissues at the site of infection. Romer⁸ found that the tissues of the conjunctiva could apparently produce antiabrin, that is, developed a tolerance for abrin ascribed to the production of an antitoxin for this substance. Hektoen,⁹ however, could find no evidences of local production of hemolysins for goat and rat erythrocytes following the injection of these cells into the anterior chamber of the eye of the dog or into the tissues of the pleura. Recently Klauder and myself¹⁰ observed the complement-fixing antibody of syphilis in secretions from chancres in sufficient amounts to give strongly positive Wassermann reactions before the blood-serum reacted positively, indicating a local production of antibody in syphilis which may possess some practical diagnostic value. It is highly probable that in some infections and particularly those of a chronic character, as syphilis and tuberculosis, there is some production of antibodies in the local tissues about the lesions.

Distribution of Antibodies in the Body Fluids.—The concentration of both natural and immune antibodies varies considerably in the different body fluids, largest amounts being found in the blood.

As between the plasma and serum of the blood, the investigations of Dreyer and Walker¹¹ have indicated that in the rabbit larger amounts of immune agglutinin for typhoid bacilli occur in the plasma than in the serum.

¹ Jour. Infect. Dis., 1915, 17, 415.

² Jour. Med. Research, 1915, 33, 183.

³ Jour. Med. Research, 1915, 33, 197.

⁴ Jour. Amer. Med. Assoc., 1912, 58, 477.

⁵ Jour. Path. and Bacteriol., 1911, 15, 262.

⁶ Zentralbl. f. Innere Med., 1913, 35, 953.

⁷ Archiv. Int. Med., 1920, 26, 706.

⁸ Arch. f. ophthal., 1901, 52, 72.

⁹ Jour. Infect. Dis., 1911, 9, 103.

¹⁰ Arch. Dermat. and Syph., 1922, 5, 566.

¹¹ Jour. Path. and Bacteriol., 1909, 14, 39.

In a thorough and painstaking study of this subject by Watanabe,¹ in my laboratory, it was found that both natural and immune antibodies exist in plasma and serum in equal degree. Watanabe also devoted much time and study to the question of preformed complement in the plasma, the results of his studies indicating that complement exists in the plasma in practically the same concentration as in the corresponding serum.

Investigations by Becht and Greer,² Hektoen and Carlston³ have shown that natural and immune antibodies of various kinds in the dog are present in largest amounts in the blood followed in order by the thoracic lymph, neck lymph, pericardial fluid, cerebrospinal fluid, and aqueous humor of the eye. Immunization increased the concentration of antibodies in these fluids in the same order.

Antibodies are commonly found in inflammatory exudates including blister fluid. In the secretions, as urine, saliva, tears, and milk, they are generally absent unless traces are found during periods of unusual activity, as the syphilis antibody in the milk of a syphilitic woman during the first few days of lactation. Antibodies are likewise usually absent from transudates, as pleural and ascites fluid unless there are relatively large amounts in the blood at the same time. The cerebrospinal fluid is usually free of complement and antibodies during health, and in this respect resembles other transudates; in syphilis, however, the complement-fixing and other antibodies are commonly present and, indeed, may be found in this fluid when absent from the blood, indicating the possibility of local production of these antibodies in the tissues of the central nervous system.

Chemical Nature of Antibodies.—Owing to the impossibility of obtaining antibody in absolutely isolated and pure form accurate chemical analyses have not been possible. Furthermore, they are comparatively unstable and easily altered by strong chemical reagents. Consequently, our knowledge of the chemistry of antibodies is largely based upon indirect chemical and biologic analyses, and upon reactions analogous to those of known chemical substances.

It has long been known that antibody and, notably, diphtheria antitoxin is apparently associated with certain fractions of serum and particularly the globulins, obtained by salting out methods. These methods, however, are inadequate for determining the true nature of antibodies owing to the absorption of the latter by serum proteins.

The studies of Huntoon, Masucci, and Hannum,⁴ on the nature of antibody contained in antipneumococcus serum, have proved unusually interesting and valuable; these investigators do not believe that this antibody belongs to the serum proteins, and have drawn the following conclusions on the nature of antibody from the results of their experiments:

1. The antibody molecules are of large size, not been dialysable, and indicating their colloidal nature.
2. Antibodies are not affected by trypsin over considerable periods, indicating either that they are not protein in nature, or have been racemized by the dilute alkali used, or belong to the peptid group having a carboxyl-amino linkage.
3. Antibodies are not precipitated by solutions containing little or no electrolytic content, indicating that they are not of an euglobulin nature.
4. Antibodies are not soluble in ether; therefore they are not of the lipin group.
5. Antibodies free from any gross amount of globulins are not pre-

¹ Jour. Immunology, 1919, 4, 77.

² Jour. Infect. Dis., 1910, 7, 127.

³ Jour. Infect. Dis., 1910, 7, 319.

⁴ Jour. Immunology, 1921, 6, 185.

cipitated or affected by a short exposure to 30 per cent. sodium chlorid solution, indicating that they are not of a pseudoglobulin nature.

6. Antibodies are not injured by certain dilute alkalies or acids.

7. Antibodies are not affected by temperature up to 60° C. Higher temperatures progressively destroy or alter their nature.

The Antigen-antibody Reaction; Antigen-antibody Equilibrium; Dissociation of Antigen and Antibody.—The laws governing the reactions between antigen and antibody have never been satisfactorily worked out. Numerous investigations have established certain facts bearing upon the interaction of antigen and antibody, indicating that the mechanism is one of physical chemistry and particularly of colloidal reactions.

When antigen and antibody are brought together in solution under proper conditions, there is an immediate tendency for union which may bring about demonstrable physical changes, as in the precipitin and agglutination reactions. In other words, antigen and antibody cannot coexist *in vitro* without uniting, although the union may not destroy the identity of either. For example, apparently neutral mixtures of diphtheria toxin and antitoxin when injected into animals may result in the dissociation of toxin capable of exercising antigenic functions, which is the basis of von Behring's method of active immunization in diphtheria. Weil¹ has shown that a precipitate formed by the interaction of serum antigen and precipitin may be dissociated by various means *in vitro* with the recovery of both antigen and antibody. Similar studies by Bordet,² Morgenroth,³ Muir,⁴ and Landsteiner⁵ have shown that hemolysins and agglutinins already in union with corpuscles may act upon fresh corpuscles, indicating that fixed antibody may attack fresh antigen.

Such studies indicate, therefore, that antigen and antibody do not exist together in the blood in a free state; however, they may coexist in some form of loose combination easily dissociated both *in vivo* and *in vitro* without destroying the identity of either. Whether or not they may coexist without union in body cells is an open question upon which there are no established facts to warrant discussion.

As previously stated, the union of antibody with antigen may be broken up *in vitro* by certain procedures with the recovery of antigen or antibody, or both, in apparently unchanged condition. Weil has experimented very extensively upon this question of equilibrium and dissociation of antigen and antibody, employing the precipitin and anaphylactic reactions. He has shown that antigen and antibody may be recovered from precipitate by extractions with salt solution, solutions of sodium carbonate, and with trypsin and leukocytes. Gay and Chickering⁶ and Chickering⁷ have utilized similar principles for concentrating antipneumococcus serum by carrying down the antibodies in a precipitate for the recovery of antibody.

More recently Huntoon and Etris⁸ have studied the question of dissociation of antigen and antibody *in vitro* very extensively for the double purpose of securing antibody in solution as pure as possible free of serum constituents for chemical studies and for the preparation of antibody for therapeutic purposes in more concentrated form and free of the sensitizing action

¹ Jour. Immunology, 1916, 1, 35.

² Ann. de l'Inst. Pasteur, 1900, 14, 257.

³ Münch. med. Wchn., 1903, 1, 61.

⁴ Studies in Immunity, London, 1909, 13.

⁵ Münch. med. Wchn., 1902, xlix, 1905.

⁶ Jour. Exper. Med., 1915, 21, 389.

⁷ Jour. Exper. Med., 1915, 22, 248.

⁸ Jour. Immunology, 1921, 6, 123.

of serum. They have worked with various antigens and antibodies with special reference to the pneumococcus, and have found that various substances may dissociate antigen and antibody, as treatment with 10 per cent. saccharose solutions, 5.6 per cent. dextrose solutions, distilled water, normal salt solutions, ammonium carbonate solutions, and salt solution containing a small amount of sodium carbonate. They doubt that antipneumococcus antibody can be dissociated absolutely free; it would appear that the antibody is attached to bacterial fragments still present in the solutions, as filtration removes some antibody, although in solutions containing a small amount of alkali, as sodium carbonate, the antibody may be free, inasmuch as these may be filtered without loss of antibody. As a result of these studies Huntoon and his associates have been able to prepare solutions of protective and curative antipneumococcus antibody that contained so little protein as to yield negative or indefinite protein reactions and which failed to sensitize guinea-pigs or did so very irregularly. Furnhata,¹ working on the chemical nature of hemagglutinins, has concluded that they are colloidal substances closely associated with protein substances in serum, but are not to be considered as belonging to proteins in the ordinary sense. These results are full of promise for the improvement of serum therapy, and particularly in relation to elimination of sensitization and anaphylactic reactions by serum proteins.

Transmission of Antibodies and Immunity.—*Transmission in Colostrum.*

—The transmission of antibodies has always been a subject of much interest which has attracted considerable attention, but with conflicting statements based upon the results of experimental work. Authors have not always differentiated between the transmission of immunity and antibodies which are not entirely analogous.

Transmission of antibodies from mother to fetus is only possible through the placental circulation; after birth transmission may take place through the ingestion of colostrum and milk.

The greater number of investigations have been conducted with the agglutinins for bacteria and erythrocytes, and the results have generally been negative, that is, these antibodies have not been found in the serum of the fetus or newborn animal or have been found in much smaller amounts than in the serum or milk of the mother; this conclusion was reached by Gruenbaum,² Halban,³ Schenk,⁴ and others for human beings, and by Kraus and Loew,⁵ Park,⁶ Luedka,⁷ v. Eisler and Sohma⁸ for the lower animals. Fellenberg and Doell⁹ found that the serum of a child sometimes shows a stronger, sometimes a weaker, reaction than that of the mother, and they decline to believe in any constant ratio between them. Tunnicliff¹⁰ showed that the opsonic power of serum for various bacteria was less at birth than during adult life, and that opsonins decreased during the first month of life.

Reymann,¹¹ in a study of bacterial and hemoagglutinins in kids, found that they were absent from the serum at birth, although normally present in the blood of the mother goats. As a general rule they were present in large

¹ Japan Med. World, 1921, 1, 1.

² Münch. med. Wchn., 1897, 44, 330.

³ Wien. klin. Wchn., 1900, 13, 545.

⁴ Monat. f. Geburtsh., 1904, 19, 159, 344, 568.

⁵ Wien. klin. Wchn., 1899, 12, 95.

⁶ Proc. Soc. Exper. Biol. and Med., 1903, 1, 19.

⁷ Centralbl. f. Bakteriol., orig., 1904, 37, 288, 418.

⁸ Wien. klin. Wchn., 1908, 21, 684.

⁹ Ztschr. f. Geburtsh., 1913, 75, 285.

¹⁰ Jour. Infect. Dis., 1910, 7, 698.

¹¹ Jour. Immunology, 1920, 5, 227.

amounts in the colostrum, from which it would appear possible for them to be transmitted to the young by nursing. Howell and Eby¹ found that the offspring of immunized rabbits, as a rule, have antibodies in their serum which persist in appreciable but decreasing amounts for four to six weeks.

From these investigations it would appear, therefore, that normal or natural antibodies are not usually transmitted from mother to offspring during intra-uterine life; immune antibodies produced in the mother by an attack of disease or by vaccines may be transmitted, although this is the exception rather than the rule. After birth antibodies may be transmitted by colostrum and milk, although they may rapidly disappear from the blood of the young. It is well known that a mother cannot transmit immunity to smallpox, although a child may be borne immune by reason of an intra-uterine attack of this disease. Furthermore, the apparent immunity of a child of a syphilitic mother to syphilis is now believed due to infection of the child with this disease. It would appear, therefore, that placental transmission of natural and immune antibodies is a rare occurrence; when antibodies are found in the blood of the fetus or the newborn it is more likely that there has been a placental transmission of antigen with stimulation of the fetal tissues independent of those of the mother.

In diphtheria, however, there is apparently a natural transmission of antitoxin, inasmuch as the great majority of infants are immune to this disease and yield negative Schick reactions. The majority of adults are likewise immune, and presumably the mother may transmit an immunity good for at least one or two years. After that time the majority of children become susceptible to diphtheria and some later again acquire an immunity. Whether the child of a Schick positive mother is susceptible to diphtheria on the basis of the Schick reaction cannot be stated; studies to determine this point have not been made.

No doubt the colostrum is of considerable importance in relation to acquired resistance of the newborn. Little and Orcutt² have recently shown that the blood of newborn calves did not contain agglutinins for *Bacillus abortus* until after colostrum had been taken. Howe³ and Orcutt and Howe⁴ have further shown that certain of the blood proteins are missing in calves until they have ingested colostrum containing these globulins. This tends to show that both protective and nutritive principles in colostrum are absorbed, and probably colostrum and the mother's milk are of considerable importance in this relation not only as foods, but as transmitters of resistance against infection and of vitamins concerned in nutrition. Smith and Little⁵ have further shown that the calf deprived of colostrum lacks something that prevents intestinal bacteria from invading the body and multiplying in the various organs, especially bacilli of the colon group regarded as producing diarrhea ("scours") and multiple arthritis. Smith and Little have found these infections more common among calves deprived of colostrum, and they conclude that the function of the colostrum is essentially protective against miscellaneous bacteria that are harmless later on when the protective functions of the calf have become more effective.

Immunity may be transmitted: (1) By the placental passage of antibodies; (2) by the passage of antibodies in colostrum or milk; (3) by the active immunization of both mother and offspring by the same immunizing

¹ Jour. Infect. Dis., 1920, 27, 550.

² Proc. Soc. Biol. and Med., 1922, 19, 331.

³ Jour. Biol. Chem., 1921, 49, 115.

⁴ Jour. Exper. Med., 1922, 36, 181.

⁵ Jour. Exper. Med., 1922, 36, 181.

agent, or (4) by direct transmission in the germ plasma of the parents. The latter is probably the mechanism concerned in the transmission of the natural immunity of races and species to certain diseases, and of which we know little or nothing of the underlying principles.

The Influence of Nutrition, Drugs, and x-Rays Upon Antibodies and Immunity.—The influence of nutrition upon immunity is particularly well seen among the young, the child being nourished with mother's milk generally escaping the numerous infections to which the undernourished bottle-fed baby is subject. Errors in diet among artificially fed children may readily favor infection not only of the intestinal tract, but of the skin and other tissues as well, although the function of antibody production may not be interfered with as indicated by the results of experiments conducted by Hektoen,¹ who found that rats fed on pure vegetable proteins (the stunting food of Osborne and Mendel) reacted to antibody production about as well as fully nourished rats.

Previous reference has been made to the influence of drugs upon phagocytosis (see page 129).

Numerous investigations within recent years have indicated that certain drugs may induce a state of temporary immunity to trypanosome infections by stimulating the antibody-producing tissues, the leukocytic mechanism, or both, or combine with antibodies and render the latter more active.

Ehrlich and Shiga² have shown that mice infected with caderas and treated with one or more injections of trypan-red, developed a temporary immunity which could not be ascribed to an antibody response following infections with the parasites alone or to the presence of unexcreted dye, but rather to the presence of antibodies in response to the stimulating influence of the drug; later Ehrlich³ demonstrated the same phenomenon with *T. brucei* and Halberstaedter⁴ in similar studies found the immunity highly specific, that is, mice infected with dourine and treated with trypan-red developed an immunity to dourine alone and not to other trypanosomes, as *T. brucei* or vice versa. Corroborative evidence of the apparent effect of this and other drugs upon antibody production was given later by the extensive work of Terry,⁵ who found that a strong immunity against surra of India was obtained by injecting mice with dyes either alone or in combination with acetylatoxyl. That the action of the drugs is indirect rather than wholly trypanocidal, was seemingly shown by the fact that large intraperitoneal injections of surra and caderas were capable of infecting mice when introduced as early as twenty-four hours after the drug and before the latter had been wholly excreted.

Further indications of the possible important relation of drugs to immunity is shown in the reports of several homeopathic physicians, as in that Watters,⁶ who claimed that the administration of calcium sulphid increased the opsonic index to staphylococci; of Mellon,⁷ who found that the administration of baptisia influences favorably the production of group agglutinins for typhoid and other closely related bacteria and that veratrum viride increased the opsonic index to pneumococci; of Wheeler,⁸ who claims

¹ Jour. Infect. Dis., 1914, 15, 245.

² Berl. klin. Wchn., 1904, 41, 329, 362.

³ Berl. klin. Wchn., 1907, 44, 233, 280, 310, 341.

⁴ Centralbl. f. Bakt., orig., 1915, 38, 525.

⁵ Monograph No. 3, Rockefeller Inst.

⁶ North Amer. Jour. Homeop., 1909, 24, 460.

⁷ Med. Century, 1913, 20, 261.

⁸ Brit. Homeopath. Jour., 1914, 4, 243.

that phosphorus increases the opsonic index of human serum to the tubercle bacillus; of Wesselhoeft,¹ whose experiments were interpreted as indicating the curative effects of quinin in malaria, could not be ascribed entirely to its parasitidal activity, but probably in part to a favorable influence upon the production of antiplasmodial antibodies; and of Hooker,² who showed that the administration of phosphoric acid, arsenious anhydrid, and mercuric chlorid homeopathically to normal persons, resulted in the elaboration of agglutinins and complement-fixing antibodies for *Bacillus typhosus*, *B. paratyphosus* A and B, and *B. dysenteriae*. In several of these investigations the drugs alone were administered to healthy persons, and the appearance of an increase of certain group antibodies in the blood-serum was interpreted as an increase of normal or natural antibody, and an indication of the possible stimulating influence of these drugs upon antibody-producing tissues and a means of their curative value in certain diseases. Probably pilocarpin has received more attention than any other drug in this connection and particularly in relation to the production of diphtheria antitoxin. It is now generally accepted, however, that pilocarpin and other drugs have little or no influence upon the production of antibodies. Hajos and Sternberg³ have recently shown that pilocarpin, adrenalin, atropin, strophanthus, sodium salicylate, antipyrin, potassium chlorate, calcium chlorid, etc., have no influence upon the production of typhoid agglutinins and hemolysins in the rabbit; Joachmoglu and Wada⁴ have likewise found that atropin and pilocarpin have no influence upon the production of typhoid agglutinins by the rabbit.

Following the introduction and encouraging results of arsenical compounds in the experimental chemotherapeusis of protozoan infections, several investigators have studied their possible influence upon antibody production and particularly the influence of dioxydiamidoarsenobenzol (salvarsan), with the result that a general impression exists that part of the curative influence of dioxydiamidoarsenobenzol in spirochetes and trypanosome infections is to be ascribed to the influence of the drug in stimulating the production of protective and curative antibodies in addition to its powerful parasitidal activity. Aggazzi⁵ found that arsenious acid, atoxyl, and arsenophenyglycin increased the output of typhoid agglutinin; Friedberger and Masuda⁶ claim that salvarsan increases the content of normal agglutinins and hemolysins in the serum; Boehncke⁷ found that the administration of salvarsan may be followed by an increase of diphtheria antitoxin and of various bacteriolysins, opsonins, and precipitins, but not of complement-binding substances; Weisbach⁸ also claims that the administration of salvarsan results in an increase of agglutinin and hemolysin, while Reiter⁹ was unable to note any such influence, his experiments indicating that large doses of the drug lowers resistance to various bacteria.

As further indications of the probable important relation of certain drugs to immunity are several reports indicating that their administration may be followed by an increase of complement in the serum. Weil and Duport¹⁰ have reported that the intravenous administration of sodium

¹ New England Med. Gaz., 1913, 48, 64, 637.

² New England Med. Gaz., August, 1914.

³ Ztschr. f. Immunitätsf., 1922, 34, 218.

⁴ Arch. f. Exper. Path. and Pharmakol., 1922, 93, 269.

⁵ Ztschr. f. Immunitätsf., orig., 1909, 1, 736.

⁶ Therap. Monatschr., 1911, 25, 288.

⁷ Ztschr. f. Chemotherap., 1912, orig., 136.

⁸ Ztschr. f. Immunitätsf., orig., 1914, 21, 187.

⁹ Ztschr. f. Immunitätsf., orig., 1912, 15, 116. ¹⁰ Compt. rend. Soc. Biol., 1913, 74, 802.

bicarbonate to rabbits resulted in an increase of serum complement; Fenyvessy and Freund¹ claim similar results with the intravenous administration of calcium chlorid, and Ciuca² found that the injection of tartar emetic and salvarsan was followed by an increase of serum complement in normal and trypanosome-infected animals, while the administration of atoxyl caused a decrease of complement in the serum of normal animals and in a proportion of trypanosome-infected animals.

In the experiments by Toyama and myself,³ while massive doses of arspenamin and mercuric chlorid tended to suppress antibody production and cause a decrease in complement, smaller doses tended to increase the production of agglutinins and augment the complement content after a primary decrease.

Perhaps no drug has attracted as much attention as *alcohol* in its effects upon resistance to disease. The investigations of Müller,⁴ Wirgin,⁵ Laitinen,⁶ and others indicate that alcohol in mildly intoxicating amounts for several days after the injection of an antigen restrains the formation of antibodies. More recently Reich⁷ has observed that chronic alcoholism tends to lower the bactericidal and phagocytic activity of the blood for typhoid bacilli and to diminish the resistance of red blood-corpuscles to hypotonic salt solution. Data of this kind shows, therefore, that the prolonged administration of alcohol tends to reduce antibody production and to lower bodily resistance to disease.

As previously stated (page 148) *drugs*, as morphin, cocain, atropin, arsenic, and strychnin, do not produce antibodies. While it is common clinical experience to find addicts highly tolerant to the effects of these alkaloids, studies with their sera have generally shown that protective substances capable of neutralizing the effects of the drug are not to be found.

Since *x-rays* may have a direct and destructive action on lymphocytes, the lymphoid, and myeloid tissues, several investigators have studied the influence of these rays upon antibody production. Benjamin and Sluka⁸ found that in rabbits exposure to the *x-ray* before the injection of beef-serum diminished very much the production of precipitins; Låwen⁹ also observed that the formation of bacterial agglutinins and lysins was retarded by exposure to *x-rays*. Murphy and Ellis¹⁰ have found that after exposure to the *x-ray* mice (normal and splenectomized) became more susceptible to bovine tuberculosis than normal animals. Murphy and Taylor¹¹ found that mice exposed to the Roentgen rays become more susceptible to tumor transplants, apparently due to the action of the rays upon lymphocytes.

Hektoen¹² found that prolonged exposure of the white rat to the Roentgen ray markedly reduced the production of hemolysin for sheep corpuscles, due, it was assumed, to the destructive action on the lymphatic tissues, the spleen, and the bone-marrow. Similar experiments with the dog and rabbit yielded the same results. Simonds and Jones¹³ found the formation

¹ Ztschr. f. Immunitätsf., orig., 1913, 18, 666.

² Bull. d. l. Soc. Path. Exot., 1914, 7, 626.

³ Jour. Immunology, 1918, 3, 301.

⁴ Archiv. f. Hyg., 1904, li, 368.

⁵ Centralbl. f. Bakt., 1905, 38, 200.

⁶ Ztsch. f. Hyg., 1907-1908, lviii, 139.

⁷ Arch. f. Hyg., 1916, lxxxiv, 337.

⁸ Wien. klin. Wchn., 1908, 21, 311.

⁹ Mitt. a. d. Grenzgeb. d. Med. u. chir., 1909, 19, 141.

¹⁰ Jour. Exper. Med., 1914, 20, 397.

¹¹ Jour. Exper. Med., 1918, 28, 1.

¹² Jour. Infect. Dis., 1915, 17, 415.

¹³ Jour. Med. Research, 1915, 33, 183.

of agglutinins appreciably lowered after exposure to x -rays, although bacteriolysins, opsonins, and complement-fixing antibodies were less affected or not at all.

These observations harmonize with the view that antibodies are largely produced in the spleen, lymphoid tissues, and bone-marrow, as these structures suffer most directly from the action of the Roentgen ray when applied in large amounts. After these primary effects have passed away the power to produce antibodies may actually increase, due, as Hektoen suggests,¹ to regenerative changes in the spleen and lymphatic glands. Particular interest is attached to the influence of x -rays upon tuberculosis; as previously stated, Murphy found heavy exposures to increase the susceptibility of guinea-pigs, similar results being reported by Morton.² Kellert³ and Corper,⁴ however, failed to note any injurious effects from single exposures, so that the effects of the x -rays are probably in accordance to dosage and exposure. Small amounts of these rays may be beneficial, as evidenced by their favorable influence upon the treatment of tuberculosis of lymph-glands, furunculosis, and other bacterial infections, even though the rays are not bactericidal.

Influence of Temperature Upon Antibody Formation.—While exposure to cold is generally believed to be an important etiologic factor in the production of certain diseases, and especially those of the respiratory tract, only a small amount of work has been devoted to influence of temperature upon antibody production and leukocytic activity.

Graziani⁵ and Fukuhara⁶ found that chilling rabbits was usually followed by increased antibody production, while Trommsdorff⁷ and Lissauer⁸ reported just the opposite results, namely, that chilling reduced antibody production. Rolly and Meltzer⁹ and Ludke¹⁰ found that typhoid agglutinins and lysins are produced more rapidly and abundantly in rabbits that are kept overheated than in those which are kept cool. Foord¹¹ has found that chilling rabbits twice a day during a period of immunization for seven to ten minutes at 8° C. did not influence hemolysin production, although chilling was accompanied by slight increase in agglutinin production for typhoid bacilli.

Specificity of Antibodies.—Antibodies are usually specific for their antigen, and it is upon this general law that the reactions of immunity are based. It should be remembered, however, that not all antibodies are protective; the agglutinins, for instance, apparently do not injure their antigen. On the other hand, an animal may enjoy an immunity without demonstrating the presence of any antibody in the body fluids, and another animal may show antibodies generally considered as possessing protective powers, as, for example, the bacteriolysins, without necessarily being immune.

Upon what does the specificity of antibodies and immunologic reactions depend? Specificity was at first believed to depend solely upon some

¹Jour. Infect. Dis., 1920, 27, 23.

²Jour. Exper. Med., 1916, 24, 419.

³Jour. Med. Research, 1918, 39, 93.

⁴Am. Rev. of Tuberculosis, 1918, 2, 587.

⁵Centralbl. f. Bakteriöl., orig., 1906, 42, 633.

⁶Arch. f. Hyg., 1908, 65, 275.

⁷Arch. f. Hyg., 1908, lviii, 1.

⁸Arch. f. Hyg., 1907, 63, 332.

⁹Deutsch. Arch. f. klin. Med., 1909, xciv, 385.

¹⁰Deutsch. Arch. f. klin. Med., 1909, xcv, 424.

¹¹Jour. Infect. Dis., 198, 23, 159.

peculiar biologic relationship of the antigens, for it was found comparatively easy to differentiate the serum of animals of dissimilar nature by means of the precipitin and other reactions, and, as serum proteins, which seemed to be quite similar chemically, but which were obtained from unrelated species, were sharply differentiated by the biologic reactions, it was considered that the specificity must be dependent upon some principle quite apart from the ordinary chemical substances.

With the use of proteins other than serums, and especially when more or less purified proteins were employed, it has been quite firmly established that specificity depends upon chemical composition, and that *differences in species, as exhibited by their biologic reactions, depend upon distinct differences in the chemistry of their proteins* (Wells).

Pick and his co-workers have shown that two kinds of specificity exist in each protein molecule: (1) One of these is easily changed by various

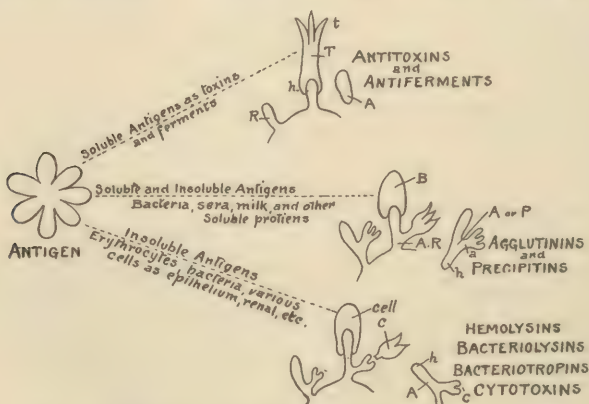


FIG. 63.—GENERAL SCHEME OF ANTIGENS AND ANTIBODIES.

Antitoxins and antiferments: *R*, Receptor of a molecule of a cell; *T*, a toxin molecule; *t*, toxophore group of the toxin molecule; *h*, haptophore group of the toxin molecule; *A*, cast-off receptor and antitoxin.

Agglutinins and precipitins: *A.R*, Receptor of cell with antigen attached; *B*, a bacterial molecule (antigen) attached to a receptor; *A* or *P*, an agglutinin or precipitin; *h*, haptophore group of the antibody; *a*, agglutinophore group of an agglutinin.

Hemolysins, etc.: *A*, Cast-off amboceptor (hemolysins, bacteriolysin, etc.); *h*, haptophore group of amboceptor; *c*, complementophil group; *C*, molecule of complement.

physical agents, such as heat, cold, and partial coagulation. When an antigen is altered by heat it produces an antibody that reacts best with the heated antigen; heating does not, however, destroy the characteristics of the antigen of this species, as its antibody will not react with the heated antigen of another species. (2) The second alteration involves a profound chemical change of the antigen, whereby it is so altered that it loses the characteristics peculiar to the species, and produces an antibody that will react with the altered antigen, but not with the unaltered antigen, even from the same animal. For example, it is possible so to alter the serum protein of a rabbit by treatment with nitric acid that the nitroprotein injected back into the same rabbit will produce an antibody specific for the nitroprotein, but which does not react with the unchanged serum protein. These changes are apparently closely related to the aromatic radicals of the protein antigen, for they are effected by introducing into the protein molecules substances that are known to combine with the benzene ring, *e. g.*, iodine, diazo- and nitro groups. Pick, appreciating the fact that the number of

different aromatic radicals in the protein molecule are limited, interprets the significance of these radicals as depending upon their arrangement, rather than upon their number, in the protein molecule. Granting the number of possible variations in the arrangement of the amino-acids in a protein molecule which the great number of these radicals provides, there is no difficulty in understanding the possibility of an almost limitless number of specific distinctions between proteins.

It may be stated, however, in general, that immunologic reactions, such as that of anaphylaxis, are as delicate in distinguishing between proteins as are chemical analyses. Distinctions may be made by these reactions with quantities too small for making accurate chemical determinations.

It may be useful here to draw up in tabular form a list of the various antigens and antibodies with which we are mainly interested in that portion of immunity involving infection with vegetable or animal parasites, and the products of their metabolism or degeneration (Fig. 63).

ANTIGENS	ANTIBODIES
Toxins:	Antitoxins:
1. Soluble bacterial toxins (diphtheria and tetanus toxins, etc.).	1. Antitoxins (diphtheria and tetanus antitoxins, etc.).
2. Phyto- (vegetable) toxins (ricin, abrin, etc.).	2. Anti- (phyto-) toxins (antiricin, anti-abrin, etc.).
3. Simple zoö- (animal) toxins (snake, spider, toad venoms).	3. Anti- (zoö) toxins (antivenins).
4. Complex zoötoxins, as snake venom, requiring complement for action.	4. Anti-hemolysins, etc.
Enzymes or ferments (rennin, lipase, etc.).	Antienzymes (antirennin, antilipase, etc.).
Precipitogenous substances (soluble animal and vegetable proteins).	Precipitins.
Agglutinogenous substances (bacteria, erythrocytes, etc.).	Agglutinins.
Opsonogenous substances (bacterial endotoxins or aggressins?).	Opsonins (acting singly or with complement).
Cytoligneous substances:	Cytolysins:
1. Vegetable cells (bacteria).	1. Bacteriolysins.
2. Animal cells (erythrocytes, spermatozoa, kidney tissue, etc.).	2. Hemolysins, spermatolysins, nephrolysins, etc.

Non-specific Immunity.—While diagnostic immunologic reactions with the body fluids are largely based upon the specificity of antibodies and the specific nature of the antigen-antibody reaction, resistance or immunity to disease may call into play certain non-specific factors which may possess considerable practical importance. Of these agencies, proteolytic ferments, leukocytosis, and the febrile reaction appear to be of most importance and especially in relation to the treatment of disease with bacterial and other proteins. Langer¹ has recently stated that such a simple procedure as the daily removal of small amounts of blood from a rabbit results in the increased production of antibodies. Olsen,² however, states that there is no actual increase to be ascribed to the effects of venesection alone, but simply the usual daily fluctuations in serum antibodies. This subject of non-specific immunity is discussed more completely in Chapter XXXIX.

Heterophile Antigen and Antibody.—The presence of antibodies in the blood is commonly ascribed to the effects of immunization with specific antigens. This is especially true of antibodies developing during disease or as a result of artificial immunization. The presence of natural antibodies in the blood, however, is much more difficult of explanation; for

¹ Ztschr. f. Immunitätsf., 1921, 31, 290.

² Ztschr. f. Immunitätsf., 1921, 31, 284.

example, the presence of hemolysin for sheep corpuscles in the sera of the majority of human beings, rabbits, and other of the lower animals. Likewise the presence of small amounts of agglutinins for typhoid and other bacilli in the sera of some human beings who have never had typhoid fever or vaccine, etc. It has been commonly stated that occult immunization was probably responsible—that the antigens gained access to our tissues in hidden and unknown ways.

The work of Forssman,¹ however, indicates another possibility. He has shown that the injection of rabbits with emulsions of the liver and kidney of the guinea-pig results in the production of antisheep hemolysin. He has designated these non-specific antigens as heterologous and the hemolysin as heterologous antibody in contradistinction to homologous antibody engendered by the immunization of rabbits with sheep corpuscles. Friedemann has suggested the terms *heterophile antigen* and *heterophile antibody*, and these terms have become more generally adopted because it is not that the antibody is generated by a different kind of antigen, but that it has an affinity for the receptors of a species other than those in response to which it was developed.

Since the work of Forssman and his associates numerous other investigators, as Orudschiew,² Rothacker,³ Doerr and Peck,⁴ Amako,⁵ Friedberger and Schiff,⁶ Sachs and Nathan,⁷ and others have discovered other heterophile antigens for antisheep hemolysin in rabbits, the subject being summarized as follows:

(a) Organs (except blood-corpuscles) of guinea-pig, horse, dog, cat, mouse, fowl, tortoise, several kinds of fish, horse urine, some bacteria, etc., contain heterophile antigens for antisheep hemolysin in rabbits and are said to be of the "guinea-pig type."

(b) Organs of ox, rabbit, pig, man, rat, goose, pigeon, frog and eel lack this property and are called by Bail and Margulies⁸ animals of the "rabbit type." Forssman originally believed that the heterophile antisheep hemolysin engendered by the injection of guinea-pig liver into rabbits was not fixed or absorbed by the emulsions of guinea-pig liver cells. Orudschiew, however, showed that absorption occurs, but that there is a relatively low affinity between the two. This view is now generally accepted and the production of heterophile hemolysin by the organs and substances mentioned above is ascribed to the wide distribution of the same or common antigenic substance for sheep hemolysin. Taniguchi⁹ and others have found that the substance in organs capable of fixing or absorbing heterophile hemolysin resides in the lipoids of these tissues, especially in those which are soluble in alcohol and ether and insoluble in acetone (the so-called lecithin fraction). The addition of cholesterol increases fixing capacity. The heterophile antibody not only combines with these lipoids but also fixes complement in their presence and forms precipitates with them. These alcohol-soluble lipoids, however, are not capable of engendering the heterophile hemolysin; apparently whole extracts of the tissues are required. For this reason Taniguchi states that the antigenic activity of the organs of the "guinea-pig type"

¹ Biochem. Ztsch., 1911, 37, 78; 1912, 44, 336; 1914, 51, 6.

² Ztschr. f. Immunitätsf., orig., 1913, 16, 268.

³ Ztschr. f. Immunitätsf., orig., 1913, 16, 491.

⁴ Biochem. Ztsch. 1913, 40, 129; Ztsch. f. Immunitätsf., orig., 1913, 19, 251.

⁵ Ztschr. f. Immunitätsf., orig., 1914, 22, 641.

⁶ Berl. klin. Wchn., 1913, 1557, 2328; Ztsch. f. Immunitätsf., 1919, 28, 217, 237.

⁷ Ztschr. f. Immunitätsf., orig., 1913, 19, 235.

⁸ Ztschr. f. Immunitätsf., orig., 1913, 19, 185.

⁹ Jour. Pathology and Bacteriology, 1921, 24, 217, 241, 456.

resides in some lipoid-protein complex, whereas combining affinity resides in the lipoids.

It is to be noted that all experiments have been conducted with rabbits and not all investigators have been sufficiently careful in controlling the factor of the natural antishoop hemolysin in rabbit-serum. Unless great care is exercised it is easy to fall into the error of regarding natural hemolysin the product of stimulation by heterophile antigens as emulsions of guinea-pig liver and kidney cells. I have found that immunization of rabbits with these substances sometimes results in the production of antishoop hemolysin, but only in rabbits known to contain natural antishoop hemolysin by preliminary titrations. With rabbits known to be free of natural hemolysin, the injection of guinea-pig liver and kidney have not resulted in the production of antishoop hemolysin. In my opinion these heterophile antigens are capable of stimulating an increased production of natural antishoop hemolysin by non-specific stimulation of the tissues concerned in the production of this substance. This possibility is strengthened by the observation that rabbits immunized with typhoid bacilli may produce typhoid agglutinin after a period of rest by stimulation with other substances, as the injection of staphylococci and various non-specific proteins. In fact, the treatment of disease with various protein substances is based in part upon the observation that various substances may stimulate the production of antibodies against bacteria in a purely non-specific manner, providing the antibody-producing tissues are previously "sensitized" or tuned to the production of these antibodies. This subject is discussed in more detail in the chapters dealing with the mechanism of non-specific protein therapy and the non-specific activities of vaccines.

Normal and Immune Antibodies.—Antibodies, as antitoxins, opsonins, agglutinins, bacteriolysins, hemolysins, and other cytolytic substances, are frequently found in the sera of persons and in the lower animals. These are designated as normal or natural antibodies in contradistinction to those produced during disease or by artificial immunization called immune antibodies. The presence of these natural antibodies has never been adequately explained, although since some are absent at birth, to be acquired later in life, infections may be responsible, and that what are regarded as natural antibodies are rather sometimes antibodies of the immune variety.

Antiantibodies.—As shown by Ehrlich and Morgenroth¹ it is possible by injecting hemolysins to produce antihemolysins which are capable of counteracting the effect of hemolysins or of neutralizing them. Bordet² found that these antihemolysins may be produced not only by immunization with hemolytic immune serum but also with normal serum of the same species, even though this normal serum contains no corresponding amboceptors. Ehrlich has explained this phenomenon by showing that antiamboceptors act against the complementophil groups of all amboceptors and that a normal serum used for immunization may contain these groups. Similar data bearing upon the non-specificity of the antiamboceptors was obtained by Pfeiffer and Friedberger,³ who found that antiamboceptors obtained by immunizing with cholera serum acted also against typhoid serum. Walker⁴ has found that animals may be immunized against an immune serum, finding that they are then less capable of being protected by that serum, but with no increased susceptibility to infection.

¹ Berl. klin. Wchn., 1899, No. 22, 481; *ibid.*, 1900, No. 21, 453.

² Ann. d. l'Inst. Pasteur, 1904, No. 10.

³ Centralbl. f. Bakteriologie, 1903, 34, 70; *ibid.*, 1904, 37, 138.

⁴ Jour. Path. and Bacteriology, 1903, 8, 34.

CHAPTER IX

THE VARIOUS TYPES OF IMMUNITY

Kinds of Immunity.—As has been stated in the preceding chapters, it is generally agreed that various antibodies and other protective agencies exist, although opinions differ as to the source and relative importance of these to resistance to and recovery from various infections. Whether or not a particular antibody is derived from a certain group of cells is largely a matter of individual opinion because of the difficulty of deciding the point by actual experimental evidence. Of far more importance is a knowledge of the properties of antibodies and of the rôle they may play in the processes of immunity. It is seldom that resistance to, or recovery from, an infection is dependent upon one defensive factor: usually several agencies are operative, although one factor may predominate. For example, antitoxins are known to neutralize their respective toxins, and are of most value in combating the toxemias, such as diphtheria and tetanus; bacteriolysins cause the death of and may totally destroy their antigens, and play an important part in the recovery from infections with bacilli of the typhoid-colon and cholera groups; phagocytosis in itself is of importance in staphylococcus infections, and is of primary importance, in conjunction with the opsonins, in recovery from pyogenic infections in general; agglutinins and precipitins do not appear to have a direct inimical influence on their antigens, but are probably secondary factors, and contribute in some manner toward their destruction. Along with important non-specific factors these various antibodies are responsible for the different forms of immunity, which may now be considered in their more general aspects.

There are two kinds of immunity—natural and acquired. Acquired immunity is divided into two subvarieties: active and passive.

Antiblastic Immunity.—While immunity to disease is believed to be dependent in part upon phagocytic activity and antibodies as just described, Ascoli¹ believes that immunity may sometimes be due to forces antagonistic to the growth of the micro-organism in the body. From his studies in anthrax immunity he ascribed to antianthrax serum as antiblastic action directed against the metabolic activities of this organism. Dochez and Avery² have adopted this term “antiblastic immunity” to describe the retardation of growth of pneumococcus by antipneumococcus serum, which they believe is, in part at least, dependent upon inhibition of metabolic function, particularly the proteolytic and glycolytic functions, of pneumococci resulting in a retardation of nutritional processes and consequent inhibition of growth.

Depression or Infection Immunity.—It has been for years a matter of clinical experience that during one disease a second infection may show retrogressive changes. For example, Zupnik, Müller, and Leiner³ have shown that the malarial paroxysm in the typhoid fever patient frequently brings about either a temporary or permanent detoxication and improvement; the effect of erysipelas on tumors and of pregnancy on tumors are related phenomena.

Morgenroth, Biberstein, and Schnitzer⁴ have recently shown that mice infected with streptococci develop in four to twenty-four hours a temporary

¹ Centralbl. f. Bakteriöl., orig., 1908, xlv, 178.

² Jour. Exper. Med., 1916, 23, 61.

³ Wien. klin. Wchn., 1916, 29, 64.

⁴ Deut. med. Wchn., 1920, xlv, 337.

and relative resistance to a second injection of the same or other streptococci in doses that prove fatal for control animals; in other words, that during the existence of general streptococcus infection the animals are temporarily refractory or resistant to added injections of streptococci fatal for normal animals. Wiegand¹ and Berliner and Citron² have confirmed these results, working with chicken cholera infections of guinea-pigs.

The same phenomenon is seen in syphilis. It is generally believed that the uncured syphilitic is immune to reinfection with *T. pallidum*, although the patient is susceptible to exacerbations of his own infection. In other words, it would appear that the syphilitic develops an immunity to all other strains of *T. pallidum* but his own. This subject will be discussed in more detail in Chapter XXXIV, but is mentioned here as an example of this so-called *depression immunity*.

The mechanism of this resistance is not known. In the experimental work mentioned above it has been shown that the streptococci or chicken cholera organisms used in the superimposed infection are not killed, but may be found in the animals. The animal is simply refractory or immune for a time to its effects in a manner analogous to the production of the state of antianaphylaxis or desensitization to non-infectious protein agents. The immunity is not absolute, but only relative, inasmuch as superinfection with organisms of specially high virulence or in extra large doses may overcome the condition.

This immunity has been designated as "depression immunity" because the reaction of the body to superinfection is depressed or the activity of one disease depressed by the development of a second infection. For this condition of resistance or immunity to superinfection I believe the term "infection immunity" more appropriate. The benefit to be noted upon one disease by the development of a second, as the influence of malaria upon typhoid fever, erysipelas, and pregnancy upon tumors, etc., probably involves a different mechanism introducing the curative activity of non-specific agents, as fever, leukocytosis, and the stimulation of ferment and antibody production, discussed in more detail in the chapter on Non-specific Protein Therapy.

NATURAL IMMUNITY

Natural immunity is the resistance to infection normally possessed, usually as the result of inheritance, by certain individuals or species under natural conditions.

The mechanism of this type of immunity is very complex, and bears an intimate relation to the subject of infection, both local and general, the nature of the infecting parasite, and the presence or absence of specific antibodies in the body fluids. In many instances this type of immunity is dependent upon non-specific causes—is frequently relative and seldom absolute. For example, fowls are immune to what may be called an ordinary dose of tetanus toxin, but succumb readily to larger doses; rats are highly immune to diphtheria toxin, and readily withstand the effects of an amount equaling 1000 lethal doses for a guinea-pig, but still larger doses may prove fatal; hedgehogs possess complete or almost complete immunity for the amount of snake venom deposited in an ordinary strike, but if the venoms of several snakes are collected and injected at one time the result is fatal.

Species immunity is a type of natural immunity, best illustrated by

¹ Inaug. Dissertation, Marburg, 1920.

² Deut. Med. Wchn., 1920, xlii, 997.

the immunity of man to certain diseases of the lower animals, such as fowl cholera, swine-plague, distemper, Texas cattle fever, mouse septicemia, etc.; and, conversely, by the immunity of animals to diseases common to man, such as measles, cholera, typhoid fever, scarlet fever, chickenpox, etc. Although the close relation of man to the domestic animals furnishes ample opportunity for infection, yet a complete immunity is frequently observed.

Racial immunity is that type of natural immunity existing among members of the same species. For example, negroes are believed to enjoy immunity to yellow fever and Mongolians to scarlet fever. As a matter of fact, well-marked examples of racial immunity are extremely rare, as not infrequently the disease in question may have been acquired in early infancy in a clinically unrecognized form.

Similarly, close biologic relationship is no guarantee that animals will behave alike toward infection. For example, the white mouse is immune to glanders, the house mouse is somewhat susceptible, and the field mouse is highly susceptible. The rabbit, guinea-pig, and rat are rodents, but though the rabbit and the guinea-pig are susceptible to anthrax, the rat is largely immune. Mosquitoes, though closely related, behave differently toward the malarial parasite. The *Culex* does not carry the parasite at all, and of the *Anopheles*, one species, *Anopheles maculipennis*, is quite susceptible and well recognized as a carrier of the parasite, whereas *Anopheles punctipennis*, though closely related, is not susceptible to it.

Examples of *individual immunity*, while not infrequent, are not constant and seldom absolute. Certain persons appear to possess a definite immunity to scarlet fever and diphtheria, although they may be freely exposed; others may pass through various epidemics of other infectious diseases, such as measles, pertussis, etc., without becoming infected. I have noticed, on several occasions, that resident physicians, on service in scarlet fever wards for many months or years, having escaped infection though brought in intimate contact with severe forms of the disease, finally contracted the disease upon returning from a short vacation.

Mechanism of Natural Immunity.—Natural immunity may be due to the following causes:

1. *Various non-specific factors may prevent infection;* among these may be mentioned: (a) The integrity of the epithelium of the skin and mucous membranes; (b) activity of enzymes in the skin (see page 167), and (c) the chemical and physical action of various secretions, such as the gastric fluid, the intestinal juices, and the saliva.

2. *A particular route for the introduction of infecting microparasites may be necessary.* For example, intestinal diseases, such as typhoid fever and cholera, are usually due directly to swallowing of the infecting micro-organisms, infection in this type of disease seldom, if ever, occurring through the skin. This is probably due in part to the lowered vitality of the intestinal mucosa, together with a peculiar selective affinity of the bacteria for the cells of these tissues, aided by the biologic nature of the invading bacterium, which grows best under the more favorable cultural conditions of the intestinal canal. This *selective action* is further illustrated by the tendency of dysentery toxin to attack the intestinal mucosa when the bacilli or toxin is administered intravenously.

3. *Certain tissues appear to possess a marked local immunity to certain bacteria.* In considering examples of local immunity, various factors, such as the question of exposure, the thickness of the epidermis, and the kind and quantity of the local secretions, must be borne in mind. For example, *Trichina spiralis* affects the muscles, never the bones, and but rarely any

other tissue. Likewise, although diphtheria in the throat may spread in many directions, it seldom passes down the esophagus.

Some differences are known to exist in regard to local immunity as observed in the child and in the adult. For example, ringworm of the scalp is practically unknown among adults, whereas children under seven years of age are quite susceptible to the disease. These differences may be due to the greater susceptibility in general of young tissues to infection, and the local immunity constitutes but an index to the general rise in resisting power accompanying improvement in strength and vitality. In some cases this may be due perhaps to an actual strengthening of local tissues, as in the case of the adult vaginal mucosa, which is immune to the gonococcus, whereas the thin and immature infantile membrane is peculiarly susceptible.

In general, our knowledge of local immunity is quite incomplete. The subject is a difficult one, hence most attention has been given to the study of general immunity. A striking example of acquired local immunity may be seen in a patch of psoriasis, where the center is observed to be largely free from scales, whereas the margins are quite active.

The question of local immunity may be largely determined by various local non-specific factors, such as loss of blood-supply due to traumatism, thrombosis, tight bandaging, etc., and the action of severe irritants, tending to produce necrosis of the tissues.

4. *The importance of phagocytosis in natural immunity must be emphasized.* Micro-organisms are constantly gaining entrance to the tissues through numerous small abrasions of the skin and along the intestinal and respiratory tracts, and investigations have shown how important the wandering cells are in preventing infection, being ever on guard and ready to pick up and dispose of any injurious material. Even after mild infection has occurred, the *local inflammatory reaction* in which the phagocyte is a prominent factor may be so prompt in overcoming the invaders that the host will escape serious infection.

The natural immunity of the frog to anthrax has been shown to be partly dependent upon the activity of the leukocytes in engulfing and disposing the bacilli.

Similarly, a mild irritant may produce hyperemia and exudation or local accumulation of leukocytes, which aid in establishing a local immunity largely dependent upon phagocytosis. In this manner the intraperitoneal injection of sterile bouillon or even of salt solution may produce exudation and increase the immunity to infection.

5. *It may be that even after the introduction of a micro-organism or its toxin no harm results because of a lack of suitable receptors on the part of the body cells of the host for union with the pathogenic agent.* For example, tetanus toxin, being unbound by the cells, produces no effect on the turtle, and antitoxin is not produced. On the other hand, suitable receptors may be present that will bind the toxin, but produce no harmful effects because the body cells are *not susceptible* to the action of the microparasite or its products. Thus it is asserted that tetanus toxin has no effect upon the alligator, although the toxin is bound and antitoxin is produced by its body cells.

In other instances, a host may escape infection owing to the fact that there is a lowered affinity between a pathogenic agent and the body cells, so that but a small amount of harmful substances are bound to the body cells, and no particular harm results, whereas a larger dose, uniting with a greater number of cells, is capable of producing some disturbance.

6. *A natural antitoxin immunity may exist*, as the immunity of the alligator to tetanus toxin, just mentioned. Similarly, natural diphtheria

antitoxin may prevent infection, especially in those persons known to harbor virulent bacilli in the fauces. In such instances, however, it is difficult to exclude entirely the possibility that a previous minor infection has occurred, as natural antitoxin immunity persists much longer than the passive immunity resulting from the administration of an antitoxin serum.

Otto, who has recently investigated the content of diphtheria antitoxin in the blood of normal persons, found more than $\frac{1}{100}$ unit of antitoxin in each cubic centimeter of the blood of those who had been in close contact with cases of diphtheria without having been sick themselves; others usually had much less. Observations would tend to show that this quantity of antitoxin is generally sufficient to confer immunity to diphtheria, and the object of von Behring's method of active immunization is to induce the production of at least that much antitoxin by the body itself. Otto¹ found that diphtheria carriers, both those who had had the disease and those who had not, contained more antitoxin in their blood than did patients who had just recovered from an attack. This shows that the mere presence of bacilli in the throat is sufficient to stimulate the production of antitoxin, on which the immunity of the carrier himself would seem to depend. Undoubtedly physicians and nurses who are freely exposed to diphtheria and yet escape infection owe their safety rather to an acquired immunity the result of repeated contact with the bacilli than to a natural antitoxin immunity.

More recently Burrows and Suzuki² have studied the natural immunity of the rat to diphtheria toxin and the chicken to tetanus toxin by means of cultures of tissues; these investigators found that certain cells possessed a peculiar resistance and that antitoxins may be present in the plasma.

Coca, Russell, and Baughman³ found that the white rat can survive the injection of 1000 times the minimal lethal dose of toxin for the guinea-pig; this natural immunity was found not to be due to the presence of normal or natural antitoxin in the blood, but to the property of the cells of the rat of preventing the toxin from entering them or attaching itself to them.

7. *In some instances a natural immunity may be dependent, at least in part, upon antibacterial activity, due to the presence of bacteriolysins and bacteriotropins in the body fluids*, as, for example, that shown by the dog and the rat to anthrax. In other instances, however, a similar immunity may be observed that cannot be ascribed to the presence of antitoxins or bacteriolysins. In this type of immunity microparasites are apparently unable to sustain themselves, and proliferate in one animal, although aggressive enough in another of the same species.

8. *An immunity to infection, especially with such micro-organisms as the anthrax bacillus, which is markedly aggressive and but slightly toxic, may be due to the presence or production of antiaggressins.* This immunity would seem to depend not upon the bactericidal properties of the serum or leukocytes, nor upon the antitoxins, but on the presence of substances that prevent the micro-organisms from exercising their special aggressive forces.

9. *Finally, an immunity may exist because the parasite or other foreign cell does not obtain suitable nutrition in a host and thus fails to grow. This condition of athrepsia is responsible for what has been called athreptic immunity.* It has been more recently studied by Ehrlich, who found that upon transferring mouse cancer to the rat, the tumor grew for a short time only, or presumably until the special nutriment carried over with the tumor

¹ Deutsch. med. Wchn., March 12, 1914, 542.

² Jour. Immunology, 1917, 3, 219, 233.

³ Jour. Immunology, 1921, 6, 387.

was consumed. While there is no experimental basis for assuming that a similar condition may be present in bacterial life, yet such a cause may be operative and should be kept in mind.

The Skin in Relation to Natural Immunity; "Exophylaxis."—Mention has been made that natural immunity to some diseases may be due to the resistance offered to microbic invasion by the intact epithelial cells of the skin and mucous membranes; also to the bactericidal action of the secretions.

In addition to these protective agencies the skin is known to contain different proteolytic enzymes and especially the skin of adult human beings; these enzymes are probably protective by reason of their destructive effects upon microbes.

Hoffmann¹ has called particular attention to the part played by the skin in natural immunity, its activity in protecting against the entrance of pathogenic microbes—an *exophylaxis*—as well as the possibility of it being a source of some internal secretion or the seat of production of enzymes and antibodies playing an important rôle in recovery from disease of the internal organs. Hoffmann has made the epigrammatic statement that "the skin is the grave of the parasites" and leans to the view that in the acute exanthematous diseases the body endeavors to rid itself of the toxic substances through the skin by a process of digestion in which leukocytes and enzymes are concerned.

Bloch² has also expressed the conviction that the skin possesses an important biologic function by means of which the internal organs are protected against microbic infection. Both he and Hoffmann have drawn attention to the fact that an intoxication ensues when the function of the skin is destroyed, as by varnishing or burning; that the histologic structure greatly favors the absorption of secretions, and that in the exanthematous diseases the internal organs are spared to the degree that the eruption is manifest, with the therapeutic experience that anything increasing the eruption influences the patient in a favorable manner. The clinical fact that measles is least dangerous when the eruption is prompt and profuse and that in syphilis, involvement of the central nervous system is less likely when the cutaneous and mucous membrane lesions are well marked, are also to be mentioned in this connection. In syphilis, however, it may be that there are different strains of pallida, one, the dermatropic strain, producing marked cutaneous lesions, and a second or neutropic strain, producing early involvement of the tissues of the central nervous system.

It is apparent, however, that the skin plays an important rôle in natural immunity to infection, and it may well be that it is an organ playing an important part in the mechanism of recovery of disease of the other organs as well.

ACQUIRED IMMUNITY

Acquired immunity occurs in two distinct forms: (1) *Active* and (2) *passive*. A mixed form may exist, brought about by a combination of factors necessary for the development of the other two.

Active Acquired Immunity.—*Active acquired immunity is that form of resistance to infection brought about by the activity of the cells of a person or animal as a result of having had the actual disease in question, or as a result of artificial inoculation with a modified or attenuated form of the causative microparasite.*

The essential feature of this immunity is that the cells and tissues of persons or animals should, by their own efforts, and as a result of their

¹ Deut. Med. Schn., 1919, xlv, 1233.

² Cor.-Bl. f. schweiz. Aertze, 1914, xlv, 1377; *ibid.*, 1917, xlvii, 1259.

own active struggle against the action of a microparasite or its products, overcome these and become less susceptible to them than they were before.

This form of immunity is gained, therefore, only as the result of an active struggle between body cells and infecting agent, and this battle may be of any degree of severity, ranging from an attack of the disease itself that may threaten life, down to the most transitory and trivial reaction due to the injection of a minute dose of a mild vaccine.

Active acquired immunity may be gained: (1) By *accidental infection*, which is the most familiar form of acquired immunity, and follows an attack of an infectious disease, such as scarlet fever, measles, varicella, variola, or typhus fever; (2) *by inducing an attack of the disease by artificial inoculation*. This latter method of producing an active acquired immunity was illustrated by the ancient, obsolete, and discarded practice of smallpox inoculation, by which healthy persons were inoculated with the virus of a mild case of smallpox at a time when no epidemics existed and the person was in good general health and able to secure proper attention from the outset.

This process of immunization is used much more extensively in veterinary practice, where an occasional untoward or fatal result is of comparatively little importance if by its means an outbreak can be controlled or the great majority of the animals saved. As a rule, an attempt is made to render the induced disease as mild as possible by (a) using a small amount of infective material; (b) by inoculating it through an unusual avenue; (c) by inoculating it at a time when the animals are naturally less susceptible, or (d) by a combination of these methods. For example, Texas cattle fever, which is due to a protozoan (*Piroplasma bigeminum*) conveyed by the bites of infected ticks, may be combated by exposing calves while still milk fed to the bites of a few infected ticks. Another method consists in injecting a small amount of blood from an infected animal directly into the jugular vein. The object is to induce a mild attack of the disease. Occasionally a severe or fatal reaction occurs, but the number of these untoward results is much lower than the mortality among untreated animals.

(3) *Active immunity may also be gained by vaccination, i. e., by inoculation with a virus or microparasite or its products, modified and attenuated by passage through a lower animal (Jennerian vaccination) or by various other means, as age, unfavorable cultural conditions, heat, germicides, etc. (Pasteurian vaccination or bacterination)*. These subjects are considered more fully in the chapter on Active Immunization.

Active immunity, whether induced accidentally or artificially, may be *antitoxic*, as after recovery from diphtheria or as the result of active immunization with diphtheria toxin, as by von Behring's method; or *antibacterial*, as the immunity following typhoid fever or induced by typhoid vaccination, and largely dependent upon the presence of bacteriolysins in the circulating fluids.

During the process of active immunization an animal not infrequently fails to react to relatively large doses of toxin, and at the same time the quantity of antibody in the body fluid may decrease. This phenomenon has been explained as being due to atrophy of the receptors of the body cells (*receptoric atrophy*), whereby the toxin fails to exert its deleterious influence because it fails to unite with the body cells. It is curious, however, that the toxin is innocuous when present in a free state within the body fluids, even though unbound to the body cells; this condition is not well understood, and may be dependent upon other factors. A rest may restore the activity of the receptors and cells, a fact that is well recognized in the

immunization of horses for the preparation of antitoxin. Not infrequently a rabbit fails to produce hemolytic amboceptor if the injections of erythrocytes are too frequent. After a rest, however, the animal may react promptly with much smaller doses.

Passive Acquired Immunity.—*As the name indicates, this is a form of immunity that depends upon defensive factors not originating in the person or animal protected, but is passively acquired by the injection of serum from one that has acquired an active immunity to the disease in question.*

This is a sort of secondary immunity, acquired by virtue of having received antibodies actively formed by another animal that has had to resist the infecting agent in order to produce them. Two well-known examples of this type of serums are the diphtheria and tetanus antitoxins. These are produced by injecting horses with successive doses of the respective toxins. The horses are required to combat the effects of the toxins, and acquire an active immunity of increasing grade due to the production of antitoxins. When the animals are bled the antitoxin-laden serum, separated from the corpuscular elements, may be used for conferring an immunity in a person or another animal simply by injecting the serum, the latter receiving and enjoying an immunity in a passive manner.

Passive immunity is specific, that is, the serum of an animal immunized against one micro-organism will protect a second animal against that and against no other. This type of immunity is acquired just as soon as the immune serum has become mixed with the blood of the person or animal injected, and there is no negative phase. Hence in severe infections our hopes of specific therapy rest on the production of passive immunity. No matter how sick the recipient may be, under ordinary circumstances the immune serum produces no further disturbance than would be expected from the injection of a normal serum. The recipient's body cells have no additional burdens, or very slight ones only, to bear, and these are more than counterbalanced by the release from combat with toxic substances neutralized by the antibodies in the immune serum. Unfortunately, this field of therapy is limited, although recent discoveries are indicating the reasons for failure, and when these are eliminated, the field of usefulness will be much extended.

Passive immunity is of shorter duration than active immunity, and the former is especially indicated in prophylaxis for warding off an acute infection that has a relatively short incubation period. The degree of passive immunity is also seldom equal to that of an active immunity. The antibodies produced by our own cells are more lasting and possess higher protective value. This is an important factor in von Behring's method of immunization in diphtheria, when a small amount of toxin loosely bound to antitoxin is injected in the belief that the toxin becomes dissociated and serves to stimulate our body cells into producing our own antitoxin.

Passive acquired immunity is usually *antitoxic*, as, for example, that induced by the administration to man of diphtheria antitoxin prepared by the body cells of the horse. Antibacterial serums may likewise induce a passive immunity, as, for instance, that used in immunization against plague.

It is evident, therefore, that the processes whereby infections are overcome and immunity is conferred, and the general reactions that follow the introduction into the body of modified antigens in the practice of immunization, are complex processes, and in none is one antibody produced or solely responsible for the resulting immunity. The properties and action of the known antibodies are considered in subsequent chapters, particular attention being given to methods for determining their presence in the body fluids, which serve as an aid to the diagnosis of infection as based

upon the general law that the antibody is specific for its antigen, and so, when the presence of an antibody is demonstrated, it may be assumed that the antigen is or has been present.

Nothing is known concerning the nature of the immunity that is acquired against several infections, such as scarlet fever, measles, smallpox, etc., nor will much be known until the causes that give rise to these conditions have been discovered.

Theory of Vaughan.—According to Vaughan, the inability of a bacterial cell to grow in the animal body either because it cannot feed upon the protein of the body or because it is itself destroyed by the ferments elaborated by the body cells explains all forms of bacterial immunity, either natural or acquired. Thus in antitoxin immunity the toxin is regarded as a ferment that splits up the proteins of the body cells, setting the protein poison free. The body cells react with the formation of an antiferment or antitoxin, which neutralizes the toxin and prevents cleavage. The toxin itself is regarded as harmful only in so far as it is able to set free the protein poison responsible for the symptoms of the infection.

Natural immunity to any infection, according to Vaughan's theory, is explained as being due to an inability of the infecting agent to grow in the animal body.

Acquired immunity, due to recovery from an infection or occurring as a result of vaccination, is regarded as the outcome of the development in the body, during the course of the infective process, of a specific ferment that, on renewed exposure, immediately destroys the infection. The vaccine is the same protein that causes the disease, so modified that it will not produce the disease, but yet so little altered that it will stimulate the body cells to form a specific ferment that will promptly and quickly destroy the infecting agent on exposure.

Summary of Kinds of Immunity.—The various kinds of immunity and the factors probably concerned in their production, may be summarized as follows:

- | | | |
|--------------------------|---------------|--|
| | } | 1. Due to non-specific factors: |
| | | 1. Barrier of epithelium. |
| | | 2. Various secretions. |
| | | 3. A particular route of infection may be necessary, aided by the biologic nature of the invading bacterium. |
| <i>Natural Immunity</i> | } | 2. Due to local tissue immunity and selective action of micro-parasites for certain tissues. |
| | | 3. Due to phagocytosis. |
| | | 4. Due to lack of suitable receptors of body cells for a particular bacterium. |
| | | 5. Due to natural antitoxins. |
| | | 6. Due to natural bacteriolysins. |
| | | 7. Due to anti aggressins. |
| | | 8. Due to lack of suitable food material—athrepsia. |
| <i>Acquired Immunity</i> | | } |
| | 1. Antitoxic. | |
| | } | 2. Antibacterial. |
| | | Passive |
| | | 1. Anti oxic. |
| | | 2. Antibacterial. |

CHAPTER X

OPSONINS

Historic.—Although there can be no doubt as to the importance of phagocytosis in the mechanism of recovery from infection, yet it was shown by Metchnikoff, as early as 1893, that the body fluids contained substances that greatly facilitated the phagocytic process, and that leukocytes removed from this influence were practically powerless to engulf and destroy the invading bacterium. In other words, if leukocytes and bacteria are washed free from all traces of serum and then mixed, very few of the leukocytes will be found capable of phagocytizing the bacteria, which means that *spontaneous phagocytosis* is feeble and hence of slight importance. When, however, fresh serum is added, especially the serum of an animal immunized against the micro-organism used in the experiment, phagocytosis is marked, and, indeed, most impressive. Metchnikoff attributed this difference to the influence of a substance in the serum that stimulated (*stimulins*) the leukocytes to become phagocytes, *but later researches have shown that this is probably erroneous, and that the serum facilitates phagocytosis not by exerting a stimulating influence upon the leukocytes, but by preparing the bacteria for the process by making them, as it were, more attractive to the leukocytes.*

Denys and Leclef,¹ in 1895, were among the first to demonstrate the effect of serum on bacteria in the process of phagocytosis, and the fact that the active substance was not bactericidal in action, but in the nature of a new antibody. Since Metchnikoff had shown that freshly isolated or virulent strains of bacteria were not readily phagocytized, but seemed to resist or repel the leukocytes, it was natural for these observers to suggest that the action of this substance in serum was to neutralize the exotoxins and endotoxins of micro-organisms that were regarded as responsible for negative chemotactic influences, and thus, by robbing them of at least two defensive weapons, prepare them for phagocytosis.

The subject remained in an uncertain state until 1903, when Wright,² and later Wright and Douglas, demonstrated more clearly this action of serum upon bacteria in aiding phagocytosis. Using their own modification of the technic devised by Leishman for measuring the phagocytic power of the blood, these observers first determined the direct dependence of phagocytosis upon some ingredient of the blood-serum, and further proved that this substance acts directly upon bacteria, is bound by the bacteria, and renders them more easily ingested by the leukocytes, *i. e.*, more readily phagocytizable. To this substance they gave the name *opsonin* (from *opsono*, I prepare food for). At the same time, and independently of Wright, Neufeld and Rimpau conducted similar investigations with immune serum and reached similar conclusions, but called the substance *bacteriotropin*. Since then both terms have been used—the former more frequently in English literature—and this is permissible, providing that it is understood that both are practically the same antibody, and not distinct and separate from each other.

As will readily be understood, the bacterial opsonins have been studied most extensively, but opsinins may be present in normal and immune serums

¹ La Cellule, 1895, xi, 175, Centralbl. f. Bakteriöl., Abt., 1898, 24, 685.

² Proc. Roy. Soc., 1904, lxiii, 128.

for other cells, such as erythrocytes, and these *hemopsonins* are regarded as separate antibodies, distinct from hemagglutinins and hemolysins.

Definition.—*Opsonins are substances in normal and immune serums which act upon bacteria and other cells in such a manner as to prepare them for more ready ingestion by the phagocytes.*

Properties and Nature of Opsonins.—There is considerable difference of opinion regarding the identity and probable structure of opsonins in normal and immune serums. Just as agglutinin for a bacterium, such as *Bacillus typhosus*, may be found in varying amounts in normal serum, so various opsonins for different bacteria may be found in normal serums. These normal opsonins appear more or less specific for a given bacterium, and in immune serum the specific opsonic substance for the particular bacterium or cell with which immunization has been produced is developed to a high degree. Both owe their full effect to the interaction of two substances. One of these, the common substance, is thermolabile, and destroyed by heating the serum to from 56° to 58° C. for half an hour, whereas the other more specific substance remains unaffected. The latter, in both normal and immune serums, is opsonic by itself, although in the absence of the common thermolabile substance to a less degree, and is produced anew and specifically by artificial immunization or as the outcome of spontaneous infections.

Before the exact interaction of serum and cells in phagocytosis had been made clear Metchnikoff and his students attributed phagocytosis by immune serum to the so-called "fixateurs" or "substance sensibilatrice," which in general are regarded as identical with Ehrlich's amboceptors. Dean¹ expressed the view that amboceptors may exercise the functions of opsonins, which consequently cannot be regarded as independent substances. Neufeld and Topfer² and Barratt,³ however, early showed that a serum may contain opsonin for erythrocytes without being hemolytic.

In a series of investigations Hektoen^{4,5} has clearly shown that bacterio-opsonins and hemopsonins are distinct antibodies and may be differentiated from amboceptors by variation in resistance to heat and by absorption. This view is now generally maintained.

The true nature of opsonins is difficult to understand. They have been compared by Hektoen and Rudiger⁶ to receptors of the second order, with a haptophore and a toxophore or opsoniferous group. Receptors of this order, however, are active and independent of the presence or absence of complement, whereas the opsonins, although active to some extent in the absence of complement, are far more so if a complement is present as shown by Dean,⁷ Cowie and Chapin,⁸ Eggers,⁹ Browning,¹⁰ and others.

Kolmer, Toyama, and Matsunami¹¹ have shown that the addition of guinea-pig serum (complement) to commercial antimeningococcus serum in quantities that by themselves have little opsonic effect decidedly increases opsonic activity; these results have been confirmed by Hektoen and Tunnicliffe,¹² and similar findings have been reported by Meyer,¹³ working with antipneumococcus serum. These and earlier observations indicate that opsonic sera, normal as well as immune, owe their full activity to a ther-

¹ Proc. Roy. Soc., B, 1905, 76, 506.

² Centralbl. f. Bakteriöl., orig., 1905, 38, 456.

³ Proc. Roy. Soc., B, 1905, 76, 524.

⁷ Proc. Roy. Soc., 1907, 79, 399; *ibid.*, 1905, 76, 506.

⁸ Jour. Med. Res., 1907, 17, 95, 213.

⁹ Jour. Infect. Dis., 1908, 5, 263.

¹⁰ Jour. Med. Res., 1908, 19, 201.

⁴ Jour. Infect. Dis., 1906, 3, 434.

⁵ Jour. Infect. Dis., 1909, 6, 78.

⁶ Jour. Infect. Dis., 1905, 2, 128.

¹¹ Jour. Immunology, 1918, 3, 156.

¹² Jour. Infect. Dis., 1921, 29, 553.

¹³ Jour. Infect. Dis., 1920, 27, 82.

mostable opsonin and a thermolabile complement-like substance which greatly promotes the action of the first substance.

In this respect they resemble amboceptors, or receptors of the third order, opsonins in normal and immune serums representing respectively normal and immune bacterial amboceptors. One objection to this view of their structure is their activity, however slight, when the thermolabile substance is removed by heating, unless the amboceptors are complemented by an endocomplement, as from the bacteria themselves.

At the present time, therefore, not a few observers doubt that opsonins exist as true and separate antibodies, and are inclined to regard thermolabile opsonin (largely the opsonin in fresh normal serum) as a complement, and thermostable opsonin (largely immune opsonin or bacteriotropin) as an amboceptor; it would appear that either alone, but more especially the latter, may facilitate phagocytosis to some extent. This process is, however, much more marked when both substances are acting in unison. While it is true that the bacteriolysin and opsonin content of a serum do not run parallel, our methods for measuring these are not entirely satisfactory; both intracellular and extracellular lysis may be mere differences in degree, depending upon the nature of the bacterium or the concentration of the antibodies rather than upon separate and distinct antibodies.

Specificity of Natural and Immune Opsonins.—Normal serum usually contains opsonin for many different kinds of bacteria and erythrocytes. The question whether this wide range of opsonic action is dependent on a common opsonin or on several more or less specific opsonins has been answered differently by different investigators.

The investigations of Bulloch and Western,¹ MacDonald,² Rosenow,³ and Hektoen⁴ indicate that normal human serum contains several more or less distinctly specific opsonins for various bacteria and for alien red blood-corpuscles; on the other hand; Simon,⁵ York and Smith,⁶ Russel,⁷ Axamit and Tsuda,⁸ Muir and Martin,⁹ Levaditi and Inmann,¹⁰ and Klien¹¹ have maintained on the basis of absorption tests that opsonin in normal serum for bacteria is a common opsonin and that thorough absorption of a serum with one bacterium will remove all of the normal opsonins for other bacteria.

As previously stated, heating serum reduces opsonic activity, and it would appear that this thermolabile complement-like opsonin is non-specific and removable in part or whole by heating or by absorption not only with bacteria, but likewise with charcoal, chalk, yeast, cellular debris, and other substances, as shown by Simon, Neufeld, and Hune, Levaditi and Inmann, Muir and Martin, and others. Normal serum may, however, contain various thermostable opsonins for bacteria and erythrocytes, and these appear to be specific and removable only by absorption with specific antigen.

Immune opsonins developing during the course of infection or after vaccination are thermostable and highly specific; the specificity of these has never been seriously questioned.

¹ *Lancet*, 1905, 2, 1603; *Proc. Roy. Soc., B*, 1906, 77, 531.

² *Aberdeen University Studies*, 1906, 21, 323.

³ *Jour. Infect. Dis.*, 1907, 4, 285.

⁴ *Jour. Infect. Dis.*, 1908, 5, 249.

⁵ *Jour. Exper. Med.*, 1906, 8, 651; *ibid.*, 1907, 9, 487.

⁶ *Biochem. Jour.*, 1906, 2, 74.

⁷ *Johns Hopkins Hosp. Bull.*, 1907, 28, 252.

⁸ *Wien. klin. Wchn.*, 1907, 20, 1045.

⁹ *Proc. Roy. Soc.*, 1907, 79, 187.

¹⁰ *Compt. rend. Soc. de Biol.*, 1907, 62, 683.

¹¹ *Johns Hopkins Hosp. Bull.*, 1907, 18, 245.

Properties of Natural and Immune Opsonins.—Natural opsonins or those found in normal serum are largely thermolabile, that is, easily destroyed or inactivated by heating. According to Wright and Douglas,¹ Hamilton,² and others they are practically destroyed by heating for thirty minutes at 60° C. These opsonins also deteriorate quickly and disappear after three or four days' exposure to room temperature. In other words, the general properties of normal or natural opsonins are closely similar to the complements.

As shown by Levaditi,³ Neufeld,⁴ Muir and Martin,⁵ and others, immune opsonins, on the other hand, are highly thermostabile, resist drying, and are easily preserved. They closely resemble the amboceptors in these general properties.

According to Noguchi⁶ opsonins reveal their maximum action in a medium of neutral reaction. In this respect, as well as their high resistance in the dry state to high temperatures, leads Noguchi to emphasize that opsonins have certain properties characteristic of the ferments.

Source of Opsonins.—Little is definitely known regarding the source of opsonin. Thermostabile opsonin—that which is increased by artificial immunization or during disease, and is largely in the nature of an amboceptor—is probably a product of general cellular activity, and especially of the local cells at the site of infection. Thermolabile opsonin—largely the opsonin occurring in normal serum, and in the nature of a complement—is probably a product of the leukocytes and other cells as well, as it has never been proved that the leukocytes are the sole source of the complements, as Metchnikoff would have us believe.

They may be absent from inflammatory exudates, as shown by Opie,⁷ due probably to absorption by bacteria or cellular débris. Woodhead and Mitchell⁸ have found opsonins in cows' milk and whey in slightly less than the concentration in the corresponding sera.

Susceptibility to Opsonification.—As previously stated in the chapter on Infection, not all bacteria are equally susceptible to opsonification. As a general rule, recently isolated and virulent micro-organisms resist the influence of opsonins until they have undergone culture several times. This resistance may be due to capsule formation, thickening of the ectoplasm, actual self-immunization of the bacterium, or the influence of endotoxins as a protective means against the antibodies of a host, all of these being weakened or lost upon artificial culture-media.

Effect of Opsonins on Bacteria.—We know nothing definite regarding the manner in which opsonins prepare bacteria for phagocytosis except that opsonification in itself apparently does not impair the vitality of the bacterium, in so far, at least, as its viability is concerned.

Rôle of Opsonins in Immunity.—Although the exact identity of normal and immune opsonins and their relation to other antibodies is as yet unsettled, the important relation they bear to processes of immunity is generally recognized, especially their ability in aiding resistance to infection by facilitating phagocytosis. That phagocytosis is an important factor in resistance to infection and recovery from disease cannot be denied, and the

¹ Proc. Roy. Soc., 1903, 72, 357; *ibid.*, 1904, 73, 128.

² Jour. Infect. Dis., 1908, 5, 570.

³ Ann. d. l'Inst. Pasteur, 1901, 15, 904.

⁴ Centralbl. f. Bakteriöl., 1907, 38, 456.

⁵ Proc. Roy. Soc., 1907, 79, 187.

⁶ Jour. Exper. Med., 1907, 9, 455.

⁷ Jour. Exper. Med., 1907, 9, 515.

⁸ Jour. Path. and Bacteriol., 1906, 11, 408.

importance of opsonins in the processes of immunity are in direct relation. Evidences of phagocytosis by the circulating leukocytes are only occasionally encountered, but phagocytosis by the polymorphonuclear leukocytes in the tissues and by fixed tissue-cells is a common phenomenon. Opsonins greatly facilitate phagocytosis by polymorphonuclear leukocytes and by endothelial cells as well, as shown by Manwaring.¹ They are operative in some infections more than in others, and they are especially active in those conditions in which phagocytosis is recognized as the chief defensive force, as, for example, in pyogenic infections. In these conditions their presence has been taken as a measure (*opsonic index* of the resistance of the host) and, largely through the researches of Wright and Douglas, a technic for detecting their presence, kind, and quantity in the body fluids has been devised, the method and information it yields being of value under certain limitations and in some infections. (See next chapter.)

If experiments *in vitro* may be taken as an example of what occurs *in vivo*, it must be true that leukocytes are capable of consuming an enormous number of bacteria. Experiments with washed leukocytes—those removed from the influence of serum—show that spontaneous phagocytosis is very slight. Metchnikoff declared these experiments to be untrustworthy for the reason that the various manipulations of washing injures the vitality of the leukocytes. When, however, bacteria are opsonized, that is, are exposed to a serum containing opsonins, and then are thoroughly washed, it is found that the washed leukocytes engulf enormous numbers of bacteria, showing that Metchnikoff's objection to these experiments is unwarranted. Granting, then, that what we call opsonins are substances that facilitate phagocytosis, and that phagocytosis is a process of great importance, especially in certain infections, we must conclude that opsonins play a very important rôle in immunity; in fact, they constitute the very basis of the phenomenon of phagocytosis in the broader meaning of the term.

Production of Immune Opsonins.—1. These may be produced in the same manner as the agglutinating serums, immune opsonins being readily demonstrated in the same serums. For actual diagnostic work artificial immune opsonins are seldom required, but to secure an immune serum for experimental studies on opsonins a culture of *Staphylococcus pyogenes aureus* may be used in immunizing a guinea-pig as follows:

First dose: 1 loopful of twenty-four-hour agar culture in 2 c.c.

NaCl solution heated for one-half hour at 58° C. and given subcutaneously.

Second dose: 1 loopful in 2 c.c. NaCl, heated; intraperitoneally.

Third dose: 2 loopfuls in 2 c.c. NaCl, heated; intraperitoneally.

Fourth dose: 3 loopfuls in 2 c.c. NaCl, heated; intraperitoneally.

Fifth dose: 6 loopfuls in 2 c.c. NaCl, heated; intraperitoneally.

Sixth dose: 1 agar slant in 4 c.c. NaCl, heated; intraperitoneally.

2. Bleed the animals one week after the last injection has been made.

3. Owing to its large size, *Bacillus anthracis* may be substituted. This is a spore-forming organism, and since it is dangerous unless scrupulous care in handling is exercised, it is not usually wise to employ it in experimental work.

¹ Jour. Immunology, 1916, 1, 401.

CHAPTER XI

OPSONIC INDEX

WHETHER opsonins are regarded as separate antibodies or as being identical with complements and amboceptors, a measure of their quantity and power may be of aid in formulating a diagnosis, as a guide to active immunization, and as one means of determining the potency of various immune serums used for therapeutic purposes, such as antimeningococcus and antipneumococcus serums. We are mainly indebted to Leishman, Wright and Douglas, Neufeld and Rimpau, and their co-workers for devising a technic that, however imperfect it may be according to the results obtained, has opened a new and important field for the study of immunologic processes.

Principle.—This is based upon the method devised by Wright and Douglas, whereby it was sought to determine the amount and kind of opsonin in a patient's serum by comparing the degree of phagocytosis with that occurring when normal serum was used.

Definition.—*The opsonic index is the ratio of the number of bacteria ingested by a given number of phagocytes in the presence of a patient's serum, to the number ingested by the same number of phagocytes in the presence of normal serum.*

"An equal volume of the patient's serum, measured in a capillary pipet, is mixed with an equal volume of a suspension of washed leukocytes derived from a normal blood. After this 'phagocytic mixture' has been digested for a suitable period at 37° C., film preparations are made and stained.

"A 'phagocytic count' is then undertaken, *i. e.*, the average bacterial ingest of the leukocytes in the phagocytic mixture is determined, and this is compared with the average ingest of the leukocytes in a phagocytic mixture made with normal blood.

"The expression thus obtained,

$$\frac{\text{Average ingest of the individual phagocyte in the mixture containing the patient's serum.}}{\text{Average ingest of the individual phagocyte in the mixture containing normal serum is denoted the opsonic index" (Wright).}}$$

Purpose of the Method.—The opsonic index aims to serve as a guide:

1. In diagnosing the presence of bacterial infection, or rather in discovering whether the natural protective powers of the patient's blood have been diminished or increased as the result of the immunizing influence of the infection.

2. In connection with vaccine therapy, to guard against diminishing the opsonin content of the patient's blood; to assure ourselves that our efforts to increase them have been successful, and occasionally to ascertain how long the store of opsonin that has been obtained for the patient remains in the blood.

Limitation of the Method.—In ascertaining the opsonic index of a patient's serum, we must take it for granted—although it has not been proved:

1. That the bacteria act the same in the body as they do in the test-tube. This is known not to be the case, for virulent organisms resist phagocytosis, whereas a non-virulent strain of the same bacterium is easily phagocyted. If, therefore, a laboratory culture of attenuated organisms is used in making the opsonic index, the result can hardly be accepted as a criterion of the power of the patient to overcome the "resistant" or more virulent organism as it occurs in the body. This source of error can be overcome in a manner if the micro-organism is isolated and used at once before attenuation occurs.

2. That the leukocytes are a constant factor, and need not be taken into account. Investigation has shown that, as a result of infection, the leukocytes probably undergo qualitative changes and it is hardly fair to accept phagocytosis by normal leukocytes as a criterion of phagocytosis with the patient's own leukocytes, as it occurs in the body during the infection.

3. The method assumes that phagocytosis by the polynuclear leukocyte plays a large part in overcoming the infection. In many cases, however, this is by no means proved. For example, in tuberculosis it is not this form of leukocyte, but the mononuclear form or the lymphocyte, which seems to be more important, and hence it is difficult to understand how the index with the polynuclear leukocyte can aid the question of diagnosis or treatment.

4. The chances for error are considerable. To be of any value, the work requires experience and painstaking care. The results obtained by competent workers with the same blood may show variation, but it must be said that, with strict attention to technic and insistence upon perfect preparations, the worker may usually obtain valuable results. An index taken at one time by one person and later by another, and so on, will not be of as much value as when all are taken by the same worker, who brings practice, skill, and conscientious care to his aid.

Precautions in Technic.—1. Proper controls should be used. When dealing with the tubercle bacillus, the staphylococcus, or any other saprophyte of the external surfaces, or with any pathogenic organism with which we have normally no relations, the serum of a normal individual or the mixed serum of a number of normal persons will furnish the standard of comparison. When, on the other hand, we are dealing with intestinal bacteria or with a saprophyte of the mucous membrane, where, as a rule, any relation with them will be denied, it is difficult to establish a standard of health. Pooled serum is, therefore, necessary, and will furnish a standard for comparison for the purpose of measuring the fluctuations that may occur in the patient's blood.

2. A reasonable degree of phagocytosis should occur in the control serum. This is one of the main drawbacks to the value of the method for certain pathogenic organisms, as the pneumococcus, meningococcus, streptococcus, etc., may resist phagocytosis in normal serum, and thereby show abnormally high indices with immune serum.

3. Efforts at spontaneous phagocytosis should be suppressed in order to measure more accurately the opsonin, as shown by the degree of phagocytosis independent of the inherent activities of the cell itself. Spontaneous phagocytosis can largely be overcome by using 1 per cent. solution of sodium citrate such as is used for the collection of leukocytes.

4. The ingest of a sufficiently large number of phagocytes should be counted. As a general rule, 100 cells should represent the minimum.

TECHNIC

The necessary constituents for making the test are as follows:

1. The patient's serum and normal serums to serve for the control.
2. A bacterial emulsion.

3. A suspension of washed human leukocytes in normal salt solution.

Collection of Patient's and Control Serum.—1. The blood is collected in a Wright capsule, as described in Chapter II.

2. Care must be taken not to heat the blood when sealing the tube. In drawing off the serum avoid an admixture of corpuscles, as these may lower the opsonic index.

3. If coagulation is incomplete or the serum has not been well separated, the clot may be broken up gently with a platinum wire and the tubes centrifuged. Slight discoloration of the serum from mechanically breaking up a few erythrocytes will not interfere with the test.

4. If gross contamination is avoided, blood may be kept for one or two days in a dark place without much deterioration of its opsonin content.

5. It is always well to collect the control bloods at the same time the patient's blood is taken, or, if this cannot be done, to place them in an ice-chest as soon after collection as possible. When conducting the test the control serums are pooled and mixed in a clean watch-glass.

The Bacterial Emulsion.—1. This must be perfectly uniform, free from clumps, and must not undergo agglutination, either spontaneous or with the serum to be tested.

With many bacteria, especially the motile ones, such as *Bacillus coli* and *B. typhosus*, it is comparatively easy to secure a uniform emulsion. Staphylococci, streptococci, and pneumococci, as a rule, present no difficulties. After growing the culture for from eighteen to twenty-four hours on slants of a suitable medium, add 3 c.c. of sterile 1 per cent. salt solution, and gently remove the bacterial growth with a platinum loop. The mixture is then pipeted into a separate flask or thick glass test-tube containing glass beads, and shaken by machine or by hand until it is thoroughly emulsified. If necessary, the emulsion may be centrifugalized to remove clumps and is then ready for use.

2. It must consist of bacteria that stain evenly and well.

Only young cultures should be used; for example, an eighteen- to twenty-four-hour culture of freely growing organisms and a seven days' growth of tubercle bacillus.

3. It must be of such strength as to give a leukocytic ingest that will enable adequate differentiation to be made of the opsonin content of the various serums.

An emulsion that does not yield a count of at least 250 bacteria within 100 leukocytes is too weak to yield a satisfactory differential count. If the emulsion is too thick, bacteria overlies the leukocytes and introduce error. As a general rule, a suspension containing 500,000,000 bacteria per cubic centimeter is satisfactory. Experience will teach the right density to be used, and frequent trials may be necessary before the right one is secured.

4. The bacteria must be such as will not prove seriously dangerous. In order to obviate any danger attending work with such organisms as the glanders and the tubercle bacillus, first kill the culture by pouring on a 10 to 40 per cent. solution of formalin, mix the culture, shake, transfer to a centrifuge tube, and centrifugalize until the bacteria have been carried to the bottom of the tube. Pipet off the supernatant formalin, wash in

normal salt solution, centrifuge, pipet off again, and finally mix the sediment in sufficient salt solution to make a satisfactory suspension.

The Washed Leukocytes.—These should consist mainly of the polynuclear leukocytes of a healthy person washed free from any admixture with serum. As usually obtained, the leukocytes are mixed with red corpuscles. It is necessary to collect blood for the leukocytic mixture from a person whose corpuscles are known to be insensitive to agglutination, as otherwise there is an undue lowering of the opsonic effect. *Owing to the fact that the leukocytes are likely to be altered in disease it would appear better practice to use the leukocytes from the patient, instead of those from a normal person, as described above and advised by Wright.*

1. Place 4 c.c. of sterile 2 per cent. solution of sodium citrate in distilled water in sterile 10 c.c. centrifuge tubes.

As shown by Evans¹ some of the sodium citrate on the market is of an acid reaction and, therefore, not suitable for use since leukocytes absorb H-ions from weakly acid solutions with injury to their phagocytic activities. The leukocytes may be protected if necessary by using a buffered saline solution.

2. Prick the finger and add 1 c.c. of blood. Agitate well to insure thorough mixing.

3. Centrifuge at a sufficiently high speed to mix the red corpuscles and leukocytes at the bottom of the tube and avoid clumping of the leukocytes.

4. Draw off the supernatant fluid, add 5 c.c. of 1 per cent. salt solution, mix, and centrifuge.

5. Wash once more. Draw off the supernatant fluid.

6. Add sufficient salt solution to bring the total volume up to that of the blood originally taken, *i. e.*, 1 c.c. *Mix well.*

7. The leukocytes should be used at once; as shown by Glynn, they tend to lose in phagocytic activity after standing two hours or longer. Leukocytes may also be obtained from a rabbit or guinea-pig by injecting 3 to 6 c.c. of sterile distilled water, peptone solution, bouillon, or suspension of aleuronaut into a pleural cavity and collecting the exudate after twenty-four hours.

The Test.—1. Prepare capillary pipets of approximately the same caliber. These are made by taking 6-inch lengths of soft clean glass tubing having an external diameter of $\frac{5}{16}$ inch, heating them in the middle in the tip of the blowpipe or the Bunsen flame until about $\frac{1}{2}$ inch length of tubing is quite soft. Remove from the flame, and by rapidly separating the two hands draw out the molten glass to a length of from 18 to 20 inches. After cooling, the capillary thread is cut across with a small file, so that from 6 to 8 inches is left attached to each piece of tubing. The ends must be cut square, as ragged and uneven ends are difficult to handle. By means of a



FIG. 64.—CAPILLARY PIPET FOR OPSONIC INDEX DETERMINATION.

¹ Jour. Immunology, 1922, 7, 271.

wax-pencil make a fine mark at a point about an inch from the free end of each capillary thread. This indicates the unit volume (Fig. 64).

2. Adjust a well-fitting rubber teat, and draw up the unit volume of blood-cells. A tiny bubble of air is now allowed to enter the thread, and then 1 volume of the bacterial emulsion is added; another air-bubble is allowed to enter, and finally one volume of serum, so that we have named in their order in the capillary tube from above downward, one volume of blood-cells, an air-bubble, one volume of bacterial emulsion, an air-bubble, and one volume of serum (Fig. 64).



FIG. 65.—MIXING THE CONTENTS OF A CAPILLARY PIPET.

Due precautions must be exercised to avoid the formation of bubbles.

3. By making gentle pressure on the teat these are then blown out on the surface of a clean glass slide, and perfect mixture effected by making alternate aspiration and expulsion from the capillary tube at least six times (Fig. 65).

4. Carefully reaspirate into the capillary thread, so that the mixture occupies about the middle, and seal the tip in a low Bunsen flame (Fig. 66).

5. Remove the teat, and with the wax-pencil mark the tube with the name or number of the serum.

6. A similar preparation is prepared with the pooled serum (control).

7. The phagocytic mixtures are then placed in an incubator at 37° C. for fifteen minutes, except in the case of such bacteria as the *Bacillus typhosus*

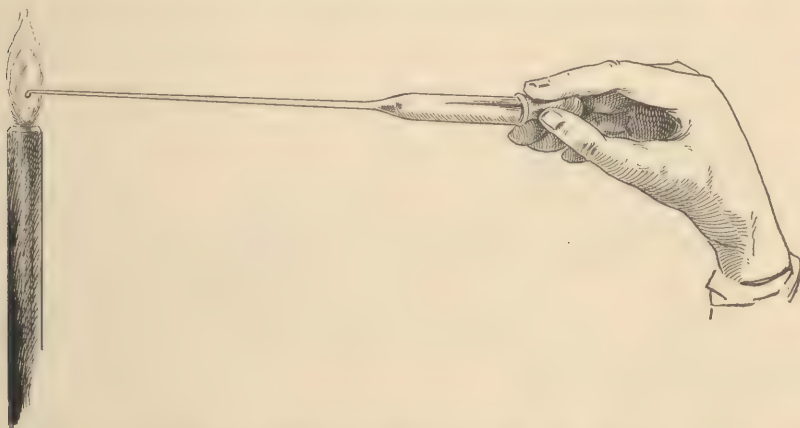


FIG. 66.—METHOD OF SEALING A CAPILLARY PIPET.

The tip of the pipet is placed in the edge of a flame. The test is held in the same position until the tip has been sealed, when it may be removed without disturbing the contents of the pipet.

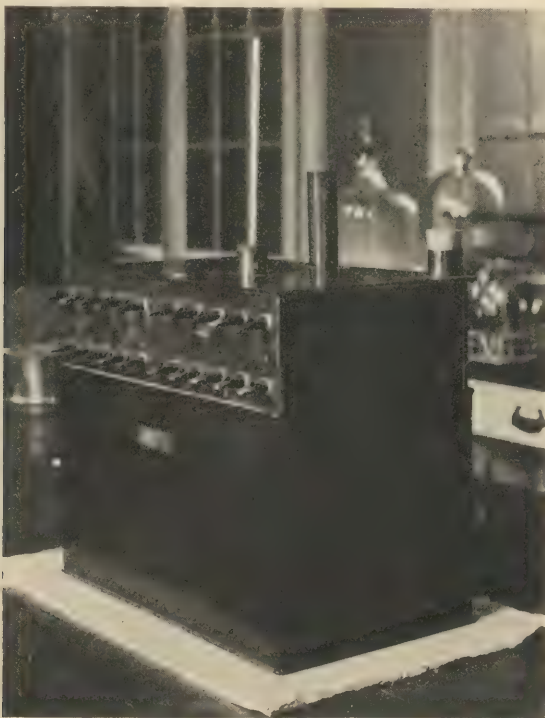


FIG. 67.—AN OPSONIC INCUBATOR.

and the *B. coli*, as lysin and agglutinin may be present in the serums of such bacteria when the period is reduced to ten minutes. The special opsonic

incubators built to accommodate individual pipets are particularly serviceable (Fig. 67).

8. The tubes are then removed from the incubator, the teats readjusted, the tip of the capillary threads scratched with a file, and evenly broken off. The phagocytic mixture is carefully expelled on a clean glass slide, and a

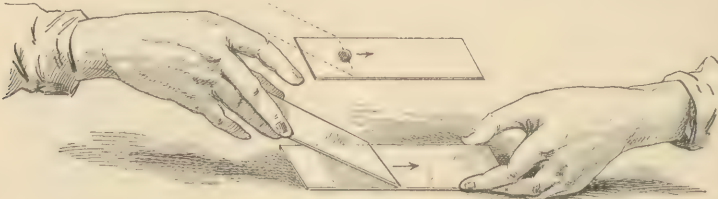


FIG. 68.—METHOD OF PREPARING A BLOOD FILM.

The slide is laid on a flat surface; the drop of blood is placed near one end; the spreader is held between the thumb and middle finger of the left hand, at an angle of about 30 degrees, and quickly *pushed* to the opposite end of the slide.

perfect mixture made by alternate aspiration and expulsion. Avoid air-bubbles. The whole is then reaspirated, and a small drop of the mixture placed on each of two clean slides that have been roughened with emery paper about $\frac{3}{4}$ inch from one extremity. With the edge of a spreader slide held at an angle of about 30 degrees, and with moderate pressure, the drop

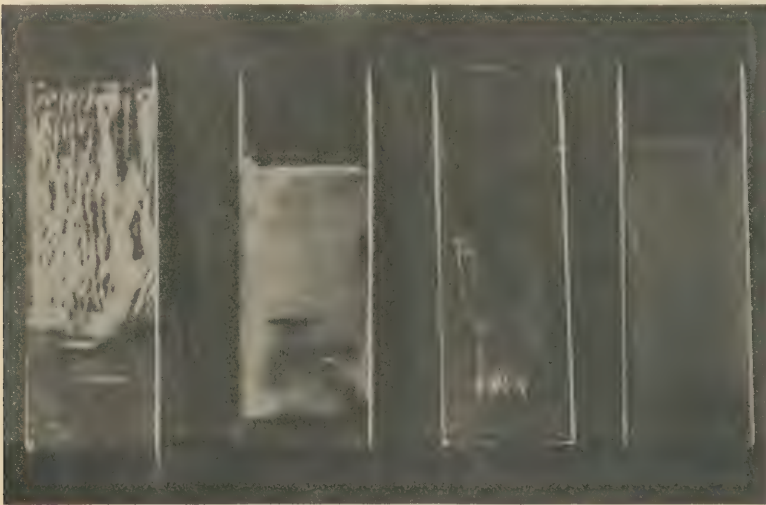


FIG. 69.—BLOOD FILMS FOR PHAGOCYTIC COUNTS.

The first slide (on the extreme left) is too thick and honeycombed, due to a greasy slide and large drop of blood; the second is likewise thick and uneven; the third is too thin, and was spread with too small an amount of blood and with the spreader held too upright; the fourth (extreme right) is a satisfactory film; it was spread on a *clean* slide, is even, smooth, and of the proper thickness.

is distributed evenly along about $1\frac{1}{2}$ inches of the surface of the slide (Fig. 68). The smears are made in duplicate, because one may be more nearly perfect (Fig. 69) than the other, or one may be spoiled in the staining, when the second may be utilized. Each slide is then labeled at one end.

9. After drying in the air, the slides must be fixed and stained.

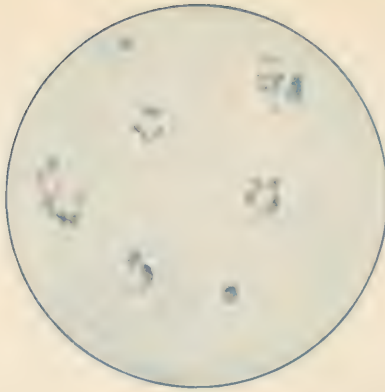


FIG. 70.—TUBERCLE OPSONIC INDEX.

A smear, stained after the method given in the text. Case IX, C. M., aged twenty-two years; active pulmonary tuberculosis; opsonic index, +1.6.

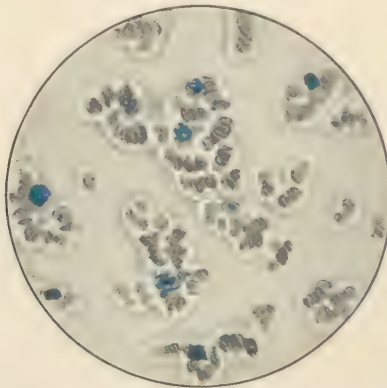


FIG. 71.—AN UNSATISFACTORY FILM FOR A PHAGOCYTIC COUNT.

Note that the leukocytes are collected in masses of erythrocytes. The slide was greasy and the smear too thick.

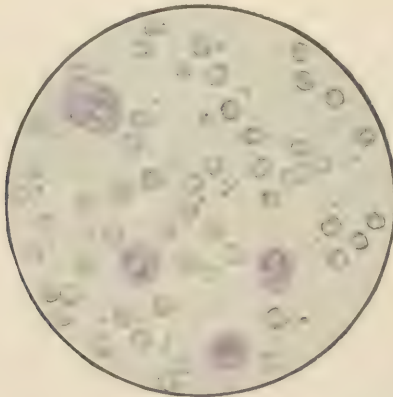


FIG. 72.—A SATISFACTORY FILM FOR A PHAGOCYTIC COUNT.

(a) **For Ordinary Bacteria.**—1. Fix by covering the slide with a saturated aqueous solution of mercuric chlorid for one minute. Wash in water.

2. Cover with carbolthionin and stain for one or two minutes. Wash in water.

3. Dry thoroughly.

(b) **For Acid-fast Bacilli.**—1. Fix by inverting the films for thirty seconds over a watch-crystal or jar containing formalin, being careful that there are no drops of formalin on the edge of the vessel that might come in contact with the preparation. The films may be fixed also with a saturated solution of mercuric chlorid or with pure methyl alcohol for two minutes. Wash in water and dry.

2. Heat a portion of carbolfuchsin almost to boiling in a test-tube, and pour the hot stain over the films. Allow to remain for at least fifteen minutes. Wash under the tap and dry.

3. Cover with a 5 per cent. solution of nitric or sulphuric acid for half a minute or longer if necessary, until decolorization is complete. Wash thoroughly under the tap.

4. Cover with 4 per cent. aqueous solution of acetic acid for one to two minutes to remove the hemoglobin from the red cells. Wash and blot lightly.

5. Cover with Löffler's methylene-blue for two minutes. Wash in water and dry thoroughly (Fig. 70).

10. Examination of the stained films with the oil-immersion objective of the microscope will show that polynuclear leukocytes have collected more toward the edges and the end at which the spreading was completed. The individual leukocytes, however, should be separated from one another (Figs. 71 and 72).

11. The edge of the film is examined, and the number of bacteria found in each series of five consecutive phagocytes is noted. If the technic has been satisfactory, no great divergence should be found in the count of each set of five cells.

12. If the films are satisfactory, divide 100 phagocytes into groups of 20. The average ingest of each group should not show a difference of over 10 per cent., otherwise the technic has been faulty and it is necessary to count 250 phagocytes or to repeat the test. If divergence is due to the fact that every now and then one cell has a considerable higher ingest than others and the bacteria are well separated, hyperactivity of the cell is probably the cause. If the bacteria are all clumped together it must be assumed that there has been a lack of care in preparing the bacterial emulsion or that agglutinin is present in the serum, and the test must be repeated with fresh precautions.

13. In opsonic work the question as to how a certain element ought to be counted becomes quite evident and important. The proper method of procedure is to determine definitely how they may best be dealt with, and then to follow the rule adopted consistently. If an organism rests on the border of a cell, it will be better to consider it as within the cell and count it. Diplococci or division forms may be counted as one or as two, provided we are consistent in our method. Individual organisms, as distinguished from zoögleic masses, which may be lying on the top of the cell, are counted as if they were within the cell; for we have no means of determining definitely whether or not our suspicions are well grounded. In the case of a beaded or vacuolated bacillus it is always better to count the whole element as a unit.

14. The *phagocytic index* is the average number of bacteria or other

cells ingested per leukocyte after counting at least from 50 to 100 cells. The total number of bacteria ingested is divided by the total number of phagocytes, the result being the average number of bacteria ingested per leukocyte, *i. e.*, the phagocytic index.

15. The *opsonic index* is then given by the ratio:

$$\frac{\text{Patient's phagocytic index}}{\text{Control phagocytic index}}$$

For example, with patient's serum, 100 phagocytes contain 300 bacteria, the phagocytic index being $\frac{300}{100}=3$. With the control serum, 100 phagocytes contain 500 bacteria, the phagocytic index being $\frac{500}{100}=5$. The opsonic index is:

$$\frac{3 \text{ Patient's phagocytic index}}{5 \text{ Control phagocytic index}} = 0.6.$$

16. Simon and Lamar have suggested a modification of Wright's method that has been adopted by many laboratories. It consists in diluting the patient's and control serums up to 1 : 10 or 1 : 100, and preparing mixtures of various dilutions with leukocytes and thinner bacterial emulsions. The percentage of phagocytic cells in the mixtures containing the serum to be tested is compared with the mixtures containing normal serum. It is, therefore, a method of comparative phagocytic indices.

Veitch Method.—A much simpler technic, which has the distinct advantage of using the patient's own leukocytes, has been described by Veitch.¹

1. The ordinary capillary pipet described is employed fitted with a rubber teat or better with a piece of rubber tubing and mouthpiece. The pipet is marked about $\frac{1}{2}$ inch from the free end in the usual manner.

2. Blood is obtained from a finger; two volumes being drawn into the pipet followed by a bubble of air, two volumes of 1.5 per cent. solution of sodium citrate, bubble of air, and one volume of bacterial suspension. The whole is immediately blown out on a clean glass slide, thoroughly mixed, and finally drawn up into the pipet the end of which is sealed in a flame.

3. The pipet is incubated for fifteen minutes, when smears are made and stained in the usual way.

4. A control is set up at the same time and in the same manner, using the blood of a healthy person, the opsonic index for the patient being calculated as described.

McC Campbell Method.—Similar methods have been described by McC Campbell² and Crane,³ employing the ordinary white corpuscle pipet. McC Campbell prepares the bacterial suspension by washing off young cultures from solid medium with small amounts of sterile 0.85 per cent. sodium chlorid, and 0.8 per cent. sodium citrate solution. The bacterial emulsion is first drawn to the mark 0.5, followed by an equal volume of patient's blood. These are mixed, redrawn into the stem of the pipet, the ends being sealed with a rubber band, and incubated for fifteen minutes, when smears are made and stained in the usual manner. The blood of a healthy person is used in the same manner as a control for the purpose of expressing an opsonic index.

QUANTITATIVE ESTIMATION OF BACTERIOTROPINS (NEUFELD)

Of the various methods for standardizing an immune serum, particularly antimeningococcus serum, and of obtaining some idea of its potency, that

¹ Jour. Path. and Bacteriol., 1908, 12, 353.

² Amer. Jour. Med. Sci., 1911, 141, 724.

³ Jour. Amer. Med. Assoc., 1909, 52.

of determining the bacteriotropic or opsonic index of the serum is in most general use.

Neufeld's¹ technic is that generally employed, and is similar to the serum dilution method employed by Simon. It varies from the technic of Wright in several particulars:

1. The immune serum is free from complement (thermolabile opsonin).

2. The actual number of bacteria within the leukocytes are not counted. Various dilutions of serum are used, and the highest dilution in which the bacteria are ingested in great numbers is compared with a normal serum in similar dilution as a control. *The highest dilution that still favors phagocytosis is then taken as the bacteriotropic titer of the serum.*

Serum.—The serum is inactivated by heating it to 55° C. for one-half hour. In old or carbolized serums this may be omitted, as they are usually free from complement. Tuberculous serums also should not be heated, as their bacteriotropins are very susceptible to heat.

Normal serum from an animal of the same species as was used in the preparation of the immune serum should be used as the normal control.

An exactly parallel series of dilutions with normal salt solution are made of the immune serum and pooled normal serums in a series of small test-tubes. At least 0.5 c.c. of each dilution should be available for the test; the following dilutions may be used: 1 : 10, 1 : 20, 1 : 50, 1 : 100, 1 : 200, 1 : 400, 1 : 600, 1 : 800, 1 : 1000, 1 : 2000, etc. After working for some time with normal serums one soon learns the dilution in which the normal bacteriotropins are attenuated. It may not be necessary, therefore, to use the whole series of dilutions with the normal serum.

Leukocytes may be obtained in several different ways: (1) By injecting a guinea-pig intraperitoneally sixteen to twenty-four hours previously with from 5 to 10 c.c. of sterile aleuronat solution. Pipet the peritoneal exudate into about 20 c.c. of sterile 1 per cent. sodium citrate in normal salt solution in centrifuge tubes. Centrifugalize, and wash the leukocytes again three times with sterile normal salt solution. The sodium citrate solution prevents the coagulation and formation of clumps of leukocytes.

2. Instead of aleuronat a sterile 25 per cent. solution of peptone may be injected in the same amount.

3. If rabbit's leukocytes are preferred, 3 to 6 c.c. distilled water or 10 c.c. of aleuronat should be injected into each pleural sac or 20 c.c. intraperitoneally. For mice, an injection of 1 c.c. of aleuronat intraperitoneally is sufficient; human leukocytes may be obtained after the method of Wright.

4. After the final washing the leukocytes are suspended in sufficient normal salt solution until an opacity equal to a 0.3 per cent. lecithin emulsion in salt solution is attained.

Culture.—Cultures should be selected with great care in order to avoid using one that displays a well-marked tendency to undergo "spontaneous phagocytosis," or, on the other hand, one unduly resistant to phagocytosis. Usually an old strain of meningococci is serviceable; it is generally necessary to try out a number of strains and select the best.

Meningococci are grown for twenty-four hours on slants of glucose agar. To each slant add 0.5 c.c. each of bouillon and of normal salt solution, and emulsify the growth. Or the bacteria may be employed in the form of a sixteen- to twenty-four-hour homogeneous broth culture. Tubercle bacilli may either be triturated in an agate mortar with 1.5 per cent. salt solution added slowly drop by drop, or the tubercle powder of Koch may be employed in an emulsion prepared in the same manner. The resultant

¹ Arb. a. d. k. Gesundheitsamte, 1907, 25, 165.

emulsion should be freed from coarser clumps by brief centrifugalization, but, as a general rule, it is very difficult to secure a uniform emulsion of tubercle bacilli by any method.

The Test.—1. The mixtures are made preferably in a series of small test-tubes about 5 cm. long and 1 cm. wide.

2. Mix 0.1 c.c. of each dilution of immune serum with 0.1 c.c. of bacterial emulsion. Plug each tube with cotton.

3. Mix and incubate for one hour.

4. Add 0.1 c.c. of leukocytic emulsion to each tube. Double this quantity may be used if the emulsion is weak.

5. Mix and incubate for from one-quarter to two hours, depending upon the variety of micro-organism.

6. At the end of the second incubation the leukocytes will have settled to the bottom of the tubes. Carefully remove the supernatant fluid from each tube; mix the sediment well with a loop, and make smears on slides. Label each slide carefully to correspond to its serum dilution.

7. Dry the smears in the air, fix with methyl alcohol, and stain with carbolfuchsin, as previously described.

The Controls.—1. A series of the lower dilutions of pooled normal serums are set up in exactly the same manner.

2. A tube containing bacteria and leukocytes without serum—to detect the extent of spontaneous phagocytosis.

Readings.—A great number of fields are examined microscopically, and a note is made of the weakest dilution that still favors phagocytosis. No attempt is made to count the phagocytized bacteria. The relative amount of phagocytosis with the immune serum in various dilutions is compared with the normal controls, and the result is expressed as the bacteriotropic titer.

Simon's Method.—This method of counting the number of empty leukocytes with a given dilution of serum is followed; a similar count is made of the normal serum control in the same dilution; thus, if in the control film 25 per cent. of leukocytes were empty, and in the patient's film, 50 per cent., the index would be $\frac{25}{50}=0.5$. A study of the results obtained by this method, and by careful counting after Wright's method, shows that they are fairly comparable, and the method may be used where it is only necessary to determine whether the index is high or low.

Hygienic Laboratory Method.—The method employed by Evans in the Hygienic Laboratory for estimating the bacteriotropins in antimeningococcus serum is described in Chapter XL.

Precautions.—1. If phagocytosis is entirely absent one should not conclude that bacteriotropins are not present. The leukocytes may have been injured, especially if heterologous leukocytes have been present; control examinations with homologous leukocytes (*i. e.*, from the same animal), as the serum should result in phagocytosis.

2. The time during which the tubes were in the incubator may have been too short or too long. Most micro-organisms require from one-half to two hours—meningococci require about one-half hour; pneumococci usually need at least two hours; typhoid and cholera bacilli about fifteen minutes to thirty minutes, as they undergo extracellular or even intracellular lysis quite readily.

3. If the control of bacteria and leukocytes alone shows well-marked phagocytosis the test should be repeated with another strain.

The method employed by Evans in the Hygienic Laboratory for estimating the tropins in antimeningococcus serum is described in Chapter XL.

PRACTICAL VALUE OF THE OPSONIC INDEX

1. In competent hands the opsonic index of normal persons to most pathogenic organisms has been found to vary from 0.8 to 1.2. As previously mentioned, it is difficult to find a perfectly normal serum for such micro-organisms as the *Bacillus coli*, the staphylococci, *B. tuberculosis*, etc., as it is unlikely that any individual can altogether escape active infection at some period of his life. As menstruation approaches, even wider fluctuations occur, the normal index being re-established by the second or third day. During the first year of life the opsonic index varies to such a degree that it has little or no practical value.

2. Although a large amount of work has been done with the opsonins in disease, it is the consensus of opinion that the determination of the opsonic index has less practical significance than was originally believed. One point is clear, however, that the work of Wright and others has broadened the field of vaccine therapy and placed it upon a firmer foundation. Aside from the 10 per cent. of chances of technical error in making an opsonin measurement, other factors may be present that are entirely beyond control and cannot be measured by the immunisator, and that may seriously affect the value of the opsonic index.

3. As a *diagnostic procedure*, Wright has shown that the opsonins possess a certain specificity, and that in a given infection a low index for a certain micro-organism indicates that this organism is probably the etiologic factor. This possibility is strengthened if the opsonic index for this micro-organism is increased by careful manipulation or exercise of the diseased part, when auto-inoculation occurs, with consequent increase of opsonin.

4. In *prognosis* the opsonic index may have some value in deciding whether an infection has been entirely overcome or is still active. An attempt is made to induce auto-inoculation, as by gentle massage of a knee-joint or a hip; active exercise; deep breathing, etc., and the index is made before, and at frequent intervals after, such attempts. If the index remains unchanged within the normal limits, the assumption is that the infection has been overcome; if, on the other hand, an increase in opsonin occurs, this indicates that an active focus remains.

5. Most value was placed by Wright upon the opsonic index as a *guide to the size and frequency of doses of bacterial vaccines* in the treatment of disease. A large number of careful determinations showed that an injection of vaccine is followed by a decrease of the opsonins (negative phase), which is of variable degree and duration, according to the amount injected (Fig. 73). This is followed by an increase (positive phase), and coincidentally there is a corresponding improvement in the patient's condition. This subject is discussed more fully in the chapter on Active Immunization.

The purpose of proper vaccination, therefore, is so to gage and time the different doses that a pronounced or prolonged negative phase is prevented as far as possible, and a high positive phase secured and maintained. It is obvious that the technic of opsonic measurement consumes much time, and that the immunisator cannot mark the index at the time a dose of vaccine is given. However, the determination of the opsonic index at proper intervals after the first dose of vaccine may give valuable information as regards the reaction of the patient, and serve as a guide to the size and frequency of subsequent doses.

As a routine measure the opsonic index has fallen into disuse, vaccine therapy being largely guided by the clinical evidences of reaction and the condition of the patient. That it has distinct value, particularly in scientific

investigation, is generally admitted, and it is well to remember that in the early years following Wright's investigations the practice of vaccine therapy was limited to those skilled in determining the index, preparing the vaccine, and carefully guiding and guarding its administration. It is to be regretted that the wholesale and indiscriminate manufacture and use of vaccines have brought this valuable field of therapy inevitably into disrepute. This

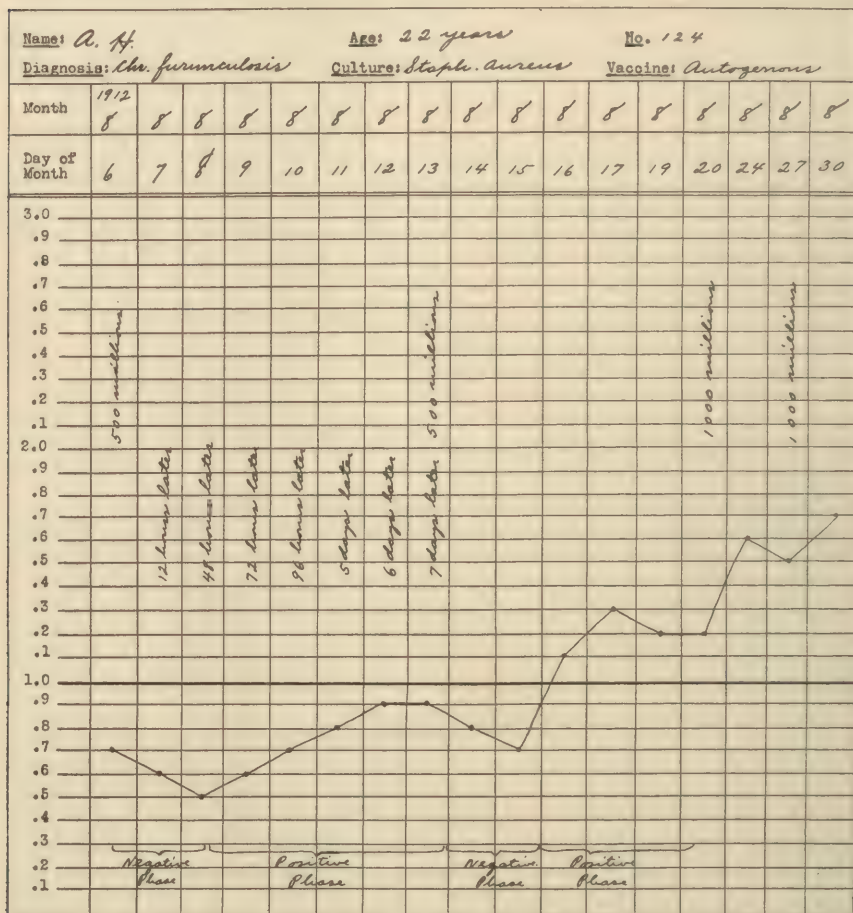


FIG. 73.—AN OPSONIC INDEX CHART.

is being realized more and more, and the effort is being made to restore the value of this form of therapy. This effort consists in recognizing the possibilities and limitations of the method, and confining its practice to those who possess at least sufficient knowledge of bacteriology to prepare a vaccine and make an opsonic measurement, the best results being secured by co-operation between bacteriologist and clinician.

CHAPTER XII

PREPARATION OF BACTERIAL VACCINES

IN this chapter a method for preparing bacterial vaccines will be described, the general discussion of vaccine therapy, with the special technic for preparing cowpox vaccine, rabies vaccine, tuberculin, and other special vaccines, being taken up in the chapter dealing with Vaccines in the Prophylaxis of Disease.

Definition.—*Bacterial vaccines are "sterilized and enumerated suspensions of bacteria which furnish, when they dissolve in the body, substances which stimulate the healthy tissues to a production of specific bacteriotropic substances which fasten upon and directly or indirectly contribute to the destruction of the corresponding bacteria"* (Wright).

TECHNIC FOR PREPARING BACTERIAL VACCINES

Bacterial vaccines are made: (1) By procuring the infected material; (2) by preparing pure cultures of the bacteria that are to be attacked; (3) by making suspensions of these in saline solution, adding a preservative, and placing in proper containers.

1. Procuring Infected Material.—Various precautions, according to existing circumstances, should be taken to avoid contamination and to secure material that is truly representative of the focal secretions. For instance, *pus* should be collected from an abscess cavity or sinus after the surrounding tissues have been cleansed with dilute tincture of iodine, for if we secured a culture of the relatively harmless *Staphylococcus epidermidis albus* from the skin instead of the *Staphylococcus aureus*, which may be the cause of infection, our vaccine will have little or no value.

Nasal secretion may be secured after cleansing the nasal orifice with soap and warm water, passing a sterile cotton swab through a nasal speculum, and rubbing the surfaces of the lower turbinates and septum lightly.

An *ear* should be cleansed, the excess of secretions removed with sterile swabs, and the culture be made of pus from the infected tissues. Various saprophytes quickly gain admission and grow in the necrotic pus, whereas the infecting bacterium is more likely to be found in the tissues.

In the collection of *sputum* special care is required: the patient should be instructed to brush the teeth with a sterilized brush, rinse the mouth several times with boiled water, and after swallowing several mouthfuls of water to cough and expectorate into a wide-mouthed sterilized bottle. The sputum may be plated at once, or washed several times in sterile Petri dishes with sterile water and then cultured.

Lung puncture may occasionally be required in infective lung conditions in which sputum is not obtainable or is too badly contaminated. A 5 to 10 c.c. all-glass syringe with a strong needle is sterilized by boiling, and 2 or 3 c.c. of peptone broth introduced. The skin of the chest wall over the site of infection, as shown by clinical evidence, is sterilized with tincture of iodine, and puncture made into the pulmonary tissues. When the desired depth has been reached, 1 c.c. of the broth is injected gently into the tissues, and after the lapse of a few seconds reaspirated as far as possible into the syringe. During the operation the patient should refrain from

respiratory movements in order to minimize any risk of lacerating the pulmonary tissues (Allen).

Urine should always be withdrawn with a sterile catheter after thoroughly cleansing the meatus. This last is especially important, for the infection may be due to a certain strain of *Bacillus coli*, and unless we are successful in obtaining a culture of this particular strain the vaccine will have little value.

Blood specimens are taken with a sterile syringe from a prominent vein at the elbow after the skin has been cleansed and sterilized with tincture of iodine, and cultured in large amounts on proper culture-media.

2. Preparing Pure Cultures.—This is frequently the most difficult step in the whole technic, for some micro-organisms, as, for example, the gonococcus and *Bacillus influenzae*, grow slowly, require special culture-media, and their colonies may easily be overlooked. To secure pure cultures, and especially to select one or at most two organisms that may be the chief offenders, considerable bacteriologic knowledge is necessary, and no simple rules or directions can be given in the limited space of this volume.

1. Stained smears of the secretions of a lesion may indicate the nature of the infection and the best culture-medium and technic to use for purposes of isolation.

2. Cultures of the lesion may be made upon solid media, and isolation carried out after a primary growth has been secured. With proper care primary cultures may be grown, such as staphylococci from the pus of a freshly incised abscess, or the micro-organism of a case of cystitis or pyelitis by securing urine with the aid of a sterilized catheter. If slowly growing organisms, such as *Bacillus influenzae*, gonococcus, pneumococcus, etc., are to be cultured, "streak" plates are usually satisfactory, and as a routine the best culture-media are, as a rule, those containing serum or blood.

3. The cultures that are to be worked up into a vaccine are usually best made on solid media. If the cultures show the presence of a single micro-organism the preparation of a vaccine is relatively simple; if more than one bacterium is present it is advisable to plate out the mixture and secure each germ in pure culture. The practice of preparing mixed vaccines by washing off the mixed growths is not to be recommended because there is no way of insuring the presence of sufficient numbers of the different bacteria, and the most important one may be present in few numbers by reason of being overgrown by the more hardy bacteria. Saprophytic bacteria should, of course, be eliminated and separate vaccines prepared of the other bacteria decided upon for incorporation in the vaccine. After each vaccine has been prepared a mixture is made in such a way that each cubic centimeter of the vaccine carries the desired number of each bacterium.

4. In making a bacterial vaccine of a freely growing micro-organism for an individual patient it will suffice to plant two agar slant tubes; when dealing with bacteria that grow much less luxuriantly, such as streptococci and pneumococci, four to six tubes should be used.

5. Cultures are usually grown for twenty-four hours at 37° C., but in the case of rapidly growing organisms a shorter period in the incubator will suffice.

6. When the cultures are ready a smear of each growth is made and stained in order to see that pure cultures of the right micro-organism were made.

7. Inasmuch as the immunizing power of a vaccine is in most cases a factor of the virulence of the organism, this being especially true of such organisms as the pneumococcus, streptococcus, and influenza bacillus, it

is well, whenever possible, to employ the first pure subculture for the preparation of the vaccine.

3. **Preparation of the Emulsion.**—Carefully observing aseptic precaution throughout, pour a portion of a test-tube of a sterile normal salt solution over the surface of the first culture, shaking the fluid in such a way

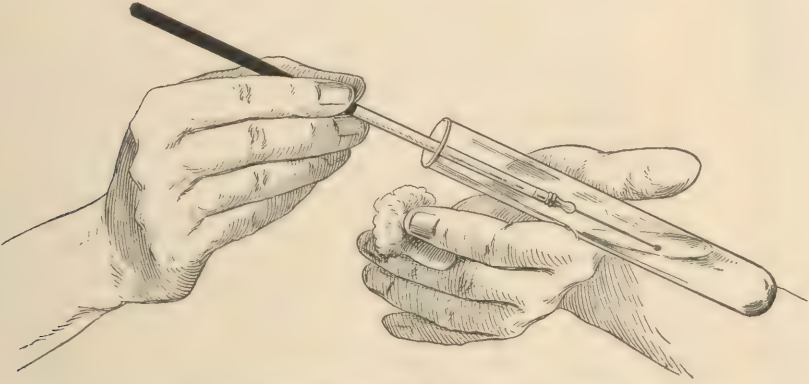


FIG. 74.—PREPARATION OF A BACTERIAL VACCINE.

Removing the culture of bacteria by *gently* rubbing over the medium (agar-agar) with a sterilized platinum loop.

as to bring the micro-organisms into suspension. If the culture is not easily washed from the medium a sterile platinum loop may be used to remove the growth, care being taken not to cut into the medium and mix the fragments with the bacterial suspension (Fig. 74).

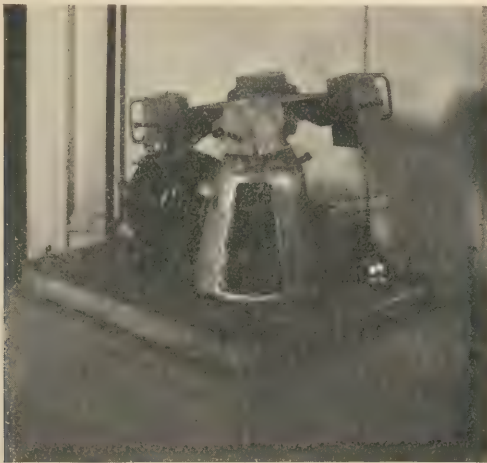


FIG. 75.—A SATISFACTORY SHAKING MACHINE MOUNTED ON A CONCRETE BLOCK.
(International Centrifuge Co.)

The bacterial suspension thus obtained is poured on the surface of the second culture, bringing this into suspension, and repeating the process until the whole series of cultures have been suspended, adding more salt solution if necessary.

The final suspension is transferred to a sterile, thick-walled flask containing glass beads, and shaken by hand or in a mechanical shaker until the bacterial masses are broken up (Fig. 75). This may be especially difficult with diphtheria bacilli and streptococci. Unless the emulsion is perfectly homogeneous, the larger particles may be removed by brief centrifugalization or, better, by filtering through a sterile filter.

There is evidence to show that bacteria grown on culture-media containing peptone may produce objectionable toxic substances capable of producing anaphylactic phenomena (Reichel and Harkins¹). In addition, when, in the preparation of a vaccine, bacteria grown on a serum medium are washed off with normal salt solution, a portion of the serum may be removed and this may be capable of producing disagreeable local and general reactions. For these reasons it is advisable to wash² all suspensions by repeated centrifugalization until the supernatant fluid reacts negatively to the biuret or ninhydrin reaction (Willard Stone).

4. Counting of the Bacterial Suspension.—Standardization, best accomplished by counting the bacterial elements contained in a unit volume of the suspension, is necessary in order to adjust our initial dose as experience will dictate and for guidance in making subsequent injections.

In dealing with a vaccine we have to count both the dead and the living bacteria, making no distinction, for both furnish the chemical agent that calls forth the elaboration of bacteriotropic substances. Inasmuch as sharp definition and the staining properties of bacteria may be lost in the process of sterilization by heat, the specimen of vaccine to be examined should be secured before sterilization is undertaken.

The counting or standardization may be done in several ways: (a) By mixing equal portions of normal blood and bacterial emulsion and counting the proportion of corpuscles to bacteria in our mixture (Wright); (b) by diluting, staining, and counting with the hemocytometer, as in the enumeration of red blood-corpuscles; (c) for standardizing large quantities of bacterial vaccine the method of Kolle or (d) that of Hopkins may be used. Nephelometric methods are also quite simple, quick, and fairly accurate.

Of these four methods probably counting by means of the hemocytometer is the most accurate and to be preferred. In the Wright method the counts are apt to be too low and the bacteria irregularly distributed in the film. Glynn, Powell, Rees and Cox,³ and Fitch⁴ have found the counting chamber method best, and especially a chamber having a depth of 0.02 mm. used for counting platelets, although the usual chamber having a depth of 0.1 mm. may be used satisfactorily.



FIG. 76.—A CAPILLARY PIPET FOR COUNTING A BACTERIAL VACCINE.

This illustration shows the pipet loaded with three equal volumes of sodium citrate solution, blood, and bacterial emulsion in proper order.

¹ Centralbl. f. Bakteriolog., etc., 1913, 69, 142.

² Jour. Amer. Med. Assoc., 1914, 63, 1011.

³ Jour. Path. and Bact., 1913, 18, 379.

⁴ Jour. Amer. Med. Assoc., 1915, 64, 893.

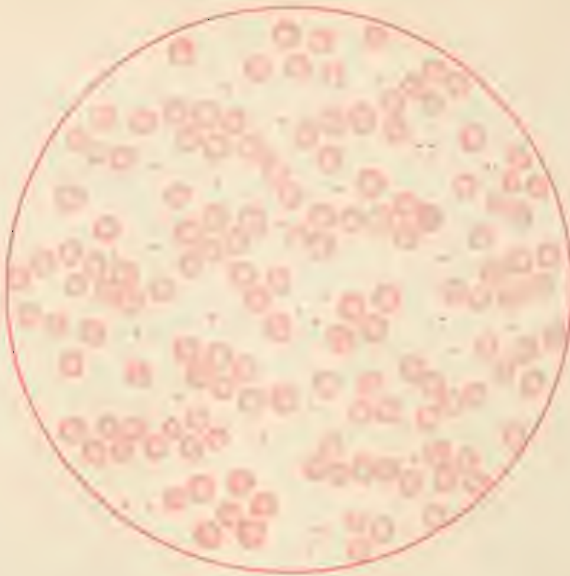


FIG. 77.—A SATISFACTORY PREPARATION FOR COUNTING A BACTERIAL VACCINE.

The micro-organisms are well separated and evenly distributed among the corpuscles; the spread is even and regular, and the corpuscles are not gathered in rouleau formation or irregular clumps.



FIG. 78.—AN UNSATISFACTORY PREPARATION FOR COUNTING A BACTERIAL VACCINE.

The micro-organisms are mostly gathered in irregular masses instead of being separated and evenly distributed; the corpuscles are not separated and evenly distributed, but show a tendency to gather in clumps; both factors render a count difficult, inaccurate, and unsatisfactory.

(a) *Method of Wright*.—Prepare a simple capillary pipet, making a mark on its stem about an inch from the tip, and fit a teat to its barrel (Fig. 59). Cleanse and prick the finger, press out a drop of blood, take up the pipet and draw up into it first one volume of sodium citrate solution, one of blood, and then either one volume of bacterial suspension or two or more volumes, if it appears on inspection to contain much fewer than 500,000,000 of bacteria to the cubic centimeter. To guard against crimping of the corpuscles in drying the films Wright advocates aspirating one or two volumes of distilled water after the blood and bacterial suspension.

Now expel from the pipet first only the distilled water and bacterial emulsion, and mix these, so that there may be no danger of the red corpuscles becoming hemolyzed, and then proceed to mix together the whole contents of the pipet, aspirating and re-expelling these a dozen times. Then make two or three microscopic films from the mixture, spreading these out on slides that have been roughened with emery.

The films are dried in the air, fixed by immersing them for two minutes in a saturated solution of corrosive sublimate, washed thoroughly, and

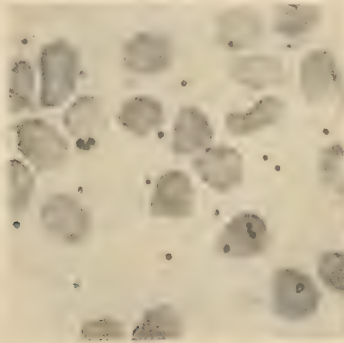


FIG. 79.—COUNTING A BACTERIAL VACCINE AFTER THE METHOD OF WRIGHT. SPECIAL OCULAR.

stained for a minute with carbolfuchsin diluted 1 : 10 or carbolthionin for two to five minutes, and then washed and dried.

The films are now given a preliminary examination. If red corpuscles and bacteria are found in approximately the same numbers and the suspension is free from bacterial aggregates, the count may be made (Fig. 77). If either the bacteria or the corpuscles are largely in excess, new mixtures and new films must be made. In case the bacteria are gathered in clumps the suspension should be shaken again and new films prepared (Fig. 78).

When satisfactory films have been obtained the actual counting may be done. This is carried out with an oil-immersion lens, and in order to secure accuracy it is necessary to restrict or divide the field by a small square diaphragm made of paper or cardboard, or by inscribing cross lines on a small clean cover-glass and dropping them on the diaphragm of the eye-piece.

A field is now chosen at random, and the corpuscles and bacteria are counted, the results being jotted down on a sheet of paper, keeping each enumeration separate and writing the numbers in two columns (Fig. 79). Proceed at random from field to field, traversing every part of the slide. Establish a rule for counting corpuscles that transgress or touch the edge of the field. Eliminate from consideration any parts of the films in which

the preparation is unsatisfactory as regards the staining, or with respect to the integrity of the red corpuscles. The examination is continued until at least 500 corpuscles have been counted, half of the count being made from the second slide. The number of micro-organisms counted is now totaled, and the approximate number per cubic centimeter estimated. Let us assume, for example, that 600 red cells and 1200 bacteria have been counted. Now, a cubic millimeter of blood contains 5,500,000 red corpuscles, and equal volumes of blood and emulsion were taken. A cubic millimeter of the emulsion, therefore, contains $\frac{5,500,000 \times 1200}{600} = 11,000,000$

organisms per cubic millimeter, or 11,000,000,000 per cubic centimeter.

(b) The *second method* of counting is precisely similar to that employed for the enumeration of blood-corpuscles, the diluting and staining fluid being made by adding to a 1 per cent. solution of sodium chlorid in distilled water sufficient formalin to make 2 per cent., and alcoholic gentian-violet, 5 per cent. The emulsion is drawn up in a white corpuscle pipet to the mark 0.5, and with diluting fluid to the mark 11. The contents are then mixed thoroughly for several minutes, several drops expelled, a drop placed in the counting chamber, and properly covered with a special thin cover-glass. The bacteria are allowed to remain in the counting cell for at least half an hour prior to enumeration. A large number of small squares are counted, and the average of one square obtained. By multiplying this figure by 4000 and then by 20, the number of bacteria per cubic millimeter is obtained, and 1000 times this figure gives the number of bacteria contained in 1 c.c. of the vaccine. If the emulsion is highly concentrated the red cell pipet may be used and the calculations made accordingly.

Callison has advised the use of the following method:

Using the red corpuscle pipet, the bacterial emulsion is drawn to 0.5 and diluting fluid to 101. Shake vigorously for three minutes, allow two or three drops of fluid to escape and load the hemocytometer chamber. After standing one-half hour the bacteria in a small square are counted or several small squares are counted and the average taken for one square. Multiplying this figure by 8 and adding eight ciphers gives the number of bacteria per cubic centimeter of vaccine.

The diluting fluid is prepared as follows:

Hydrochloric acid,	2 c.c.
Bichlorid of mercury (1 : 500),	100 "
Acid fuchsin, 1 per cent. aqueous solution, q. s. to deep cherry red.	
Filter.	

(c) *Method of Kolle*.—A platform loop adjusted to fit No. 2 of a Lautenschläger wire gage (Fig. 80) measures about 4 mm., and holds approximately 2 mg., or about 2,500,000,000 organisms. By growing an organism on slants of agar and emulsifying a certain number of loopfuls in a measured quantity of saline solution an approximate method of standardization is obtained. According to Kolle, ordinary slants of agar will hold about 15 loopfuls of staphylococci, *Bacillus typhosus*, or *B. coli*, and about 5 loopfuls of streptococci and gonococci.

(d) *Method of Hopkins*.¹—This is based upon concentrating a bacterial suspension by centrifugalization and the preparation of standard emulsions from the sediment. The emulsion is filtered through a small cotton filter to remove larger clump of bacteria and particles of agar, and is then placed in specially constructed centrifuge tubes (International Centrifuge Company, see Fig. 82), covered with rubber caps, and centrifugalized for

¹ Jour. Amer. Med. Assoc., 1913, x1, 1615.

half an hour at a speed of approximately 2800 revolutions a minute. The salt solution and bacteria above the 0.05 mark are then removed, and 5 c.c. saline solution is measured into the tube, so as to make a 1 per cent. emulsion. If the sediment does not reach the 0.05 mark, its volume is read on the scale, and a corresponding quantity of saline is added to make the emul-

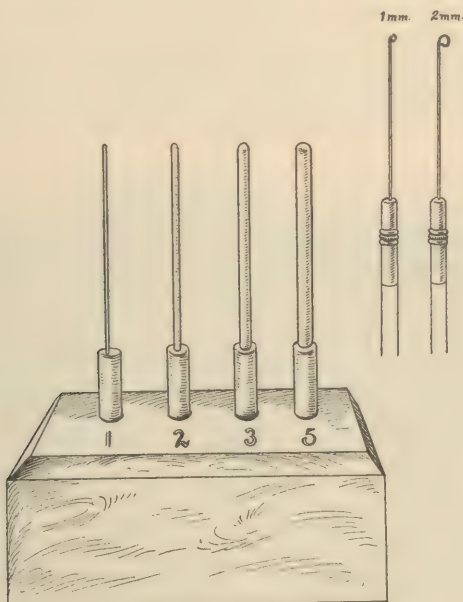


FIG. 80.—INSTRUMENT FOR THE STANDARDIZATION OF PLATINUM LOOPS.

The 2-mm. loop holds approximately 2,500,000,000 bacteria (as *Bacillus typhosus*).

sion 1 per cent. in strength. The bacteria are forced into suspension, the vaccine transferred to a sterile tube, and the micro-organisms killed in the usual manner.

Estimations of carefully counted suspensions obtained by centrifugalization in the foregoing manner gave the following results:

	PER CENT.	BILLION PER C.C.
<i>Staphylococcus aureus</i> and <i>albus</i>	1	10.0
<i>Streptococcus hæmolyticus</i>	1	8.0
<i>Gonococcus</i>	1	8.0
<i>Pneumococcus</i>	1	2.5
<i>Bacillus typhosus</i>	1	8.0
<i>Bacillus coli</i>	1	4.0

(e) *Nephelometer Methods*.—McFarland¹ has devised a useful instrument utilizing precipitates of barium sulphate for the purpose of preparing bacterial suspensions of proper density for opsonic work and in the preparation of vaccines:

Two solutions, one a 1 per cent. solution of chemically pure sulphuric acid, the other a 1 per cent. solution of chemically pure barium chlorid, are prepared, then the one added to the other so that ten standards, containing 9.9 c.c. of the sulphuric acid and 0.1 c.c. of the barium chlorid, 9.8

¹ Jour. Amer. Med. Assoc., 1907, 49, 1176.

c.c. of the sulphuric acid, and 0.2 c.c. of the barium chlorid, 9.7 c.c. of the sulphuric acid and 0.3 c.c. of the barium chlorid, etc., are prepared. According to the number of cubic centimeters of barium chlorid added, these are denominated 1, 2, 3, 4, etc. This prepares 10 c.c. of each standard solution which are sealed in small test-tubes or ampules, care being taken to use tubes of uniform diameter, and also to provide similar tubes for mixing the bacteria to be compared with the standard (Fig. 81).

With this nephelometer a vaccine of density corresponding to Tube No. 1 contains approximately 300,000,000 per cubic centimeter; density corresponding to Tube No. 3 contains about 1,000,000,000 per cubic centimeter, and density corresponding to Tube No. 5 about 1,800,000,000 per cubic centimeter, etc.

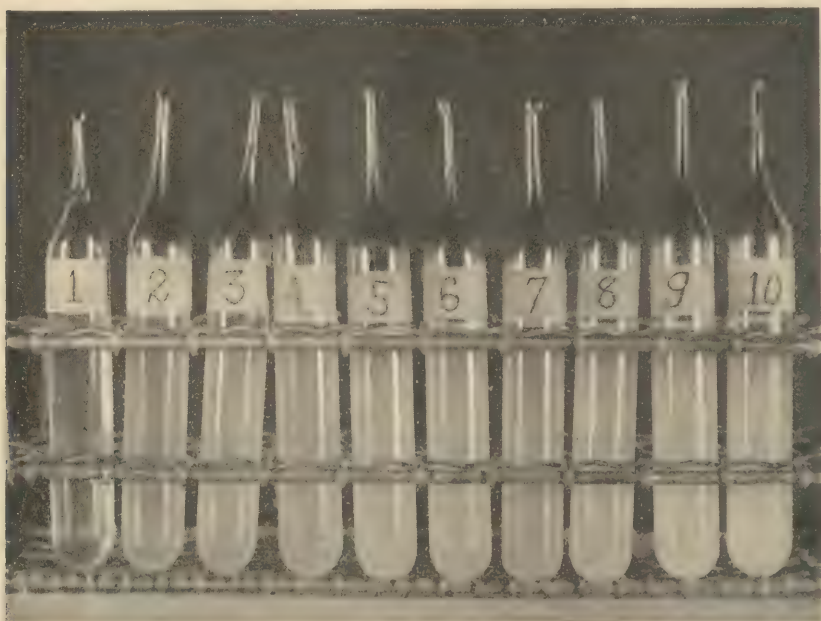


FIG. 81.—NEPHELOMETER.

Recently Dunham¹ has advised the use of the Kober nephelometer for the standardization of vaccines.

Vaccines of Fungi.—In preparing vaccines of fungi as for the treatment of ringworm, actinomycosis, and sporotrichosis, the fungus is grown on appropriate solid media, scraped off in such manner as to avoid removal of the medium, and ground in a sterile mortar with weighed amounts of crystals of sodium chlorid. Grinding should be continued until the threads are well broken up. Sterile water is gradually added until the resulting emulsion is isotonic and of a density corresponding to about Tube No. 5 of McFarland's nephelometer. The vaccine may be briefly centrifuged to remove clumps, but cannot be filtered. It is sterilized with heat and preserved with 0.3 per cent. tricresol in the usual manner.

5. Sterilizing the Vaccine and Testing its Sterility.—When, after the preliminary examination, the films for counting have been found satis-

¹ Jour. Immunology, 1920, 5, 337.

factory, a pause is made to start the process of sterilization, which may continue while the count is being made. Either heat or a germicide may be used for sterilizing vaccine, preferably the former.

The vaccine may be placed in a test-tube, which is then sealed (Fig. 83), and the whole immersed in the water-bath; a simpler method, and one just as good, is to place the flask or tube of vaccine in the bath, observing special care to see that the water is above the level of the vaccine.

Efficient sterilization is dependent upon permitting the process to continue at the minimum temperature for the minimum length of time. With the water-bath at 56° to 60° C. sterilization is nearly always complete in an hour. Boiling is likely to reduce the antigenic activity of a vaccine.¹

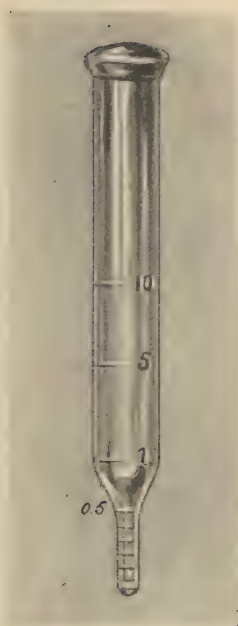


FIG. 82.—HOPKINS' TUBE FOR STANDARDIZING A BACTERIAL VACCINE.

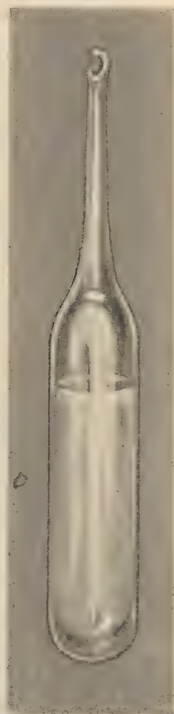


FIG. 83.—A STOCK AMPULE OF VACCINE.



FIG. 84.—STOCK BOTTLE OF BACTERIAL VACCINE.

The vaccine should be now cultured to test its sterility. At least a dozen platinum loopfuls are transferred, under strict aseptic precautions, to a slant of a suitable culture-medium, such as Löffler's blood-serum or blood-agar; this is incubated at least twenty-four hours, or longer if the organism is a slowly growing one. It is then examined, and if found sterile, the preparation of the vaccine may be completed. If not, the vaccine is heated for another hour, or, preferably, a new vaccine is prepared.

6. Dosage of Bacterial Vaccines.—As a general rule it is a good practice to so prepare a vaccine that each cubic centimeter contains the maximum number desired; in administering the vaccine the first dose may be 0.1 or

¹ Lancet, London, 1915, 2, 150.

0.2 c.c. gradually increased until the maximum amount of 1 c.c. is being given at one time. Further discussion on dosage, method of administration, and reactions is reserved for the chapter on Vaccine Therapy.

In preparing vaccines of *one micro-organism* each cubic centimeter may contain the following numbers of various bacteria:

Staphylococci.....	1,000,000,000
Streptococci.....	500,000,000
Pneumococci.....	500,000,000
Gonococci.....	500,000,000
Micrococcus catarrhalis.....	500,000,000
Bacillus typhosus.....	1,000,000,000
Bacillus paratyphosus.....	1,000,000,000
Bacillus coli.....	1,000,000,000
Bacillus pyocyaneus.....	1,000,000,000
Bacillus influenza.....	1,000,000,000

In preparing *mixed vaccines* the amounts of each micro-organism must be smaller than indicated above; *as a general rule it suffices to add equal numbers of each* so that the total will be approximately 1,000,000,000 per cubic centimeter.

7. Diluting the Vaccine and Adding a Preservative.—Having made the count and sterilized the vaccine, it is next diluted with sterile saline solution so that each cubic centimeter contains the dose decided upon. If the treatment is likely to be prolonged, a sufficient number of doses should be provided for. It is a good plan not to dilute all the vaccine, but to preserve the remainder undiluted in case larger doses are subsequently needed. If, for instance, a vaccine of *Staphylococcus aureus* contains 1,500,000,000 organisms per cubic centimeter and the dose decided upon is 500,000,000 per cubic centimeter, sufficient vaccine for 30 doses is prepared by withdrawing 10 c.c. of vaccine in a sterile container and adding 18.8 c.c. of sterile salt solution and 1.2 c.c. of a 5 per cent. solution of tricresol or phenol as a preservative. This mixture is agitated to insure thorough mixing, and 0.2 c.c. of 5 per cent. tricresol is added to each 5 c.c. of vaccine as a preservative against chance contamination. Thus, in the foregoing example, 1.2 c.c. of the diluted tricresol would be added. The amount of stock vaccine is estimated or measured, 0.5 per cent. phenol or tricresol is added, and the vaccine stored in a sterile container in the refrigerator, being first properly labeled with the patient's name, the date, and the number of bacteria per cubic centimeter.

The vaccine may now be placed in a sterile vaccine bottle, fitted with a sterile rubber cap, and properly labeled (Fig. 84). When it is to be administered, the cap is touched with tincture of iodine, the needle plunged through the cap, and a dose withdrawn with a sterile syringe. The puncture in the cap is then sealed with a drop of flexible collodion. This method is inexpensive, and with proper care is quite satisfactory, especially for stock vaccines.

Another and probably better method, especially for autogenous vaccines, consists in tubing each dose in separate sterile ampules (Fig. 85), which are then sealed in the flame. When the vaccine is to be administered, the ampule is well shaken, the neck broken in a towel, and the contents aspirated into a sterile syringe. These ampules may be purchased ready for use or be made in the laboratory, using 6 mm. soft glass tubing. For pipeting a vaccine into ampules the special automatic pipet shown in the illustration (Fig. 86) is quite convenient. As a rule, vaccines should be

preserved in a cool place, such as a refrigerator. As shown by Hitchens and Hansen¹ freezing has slight or no deleterious effects.

Deterioration of Bacterial Vaccines.—As a general rule it is highly probable that freshly prepared vaccines possess a higher immunizing power than vaccines preserved for some time; this is especially true of pertussis vaccines.

The deterioration of vaccines bears a very important relation to temperature. For example, McCoy and Bengston,² in a study of the deterioration of typhoid vaccine conducted over a period covering two and a half years, found that vaccines stored at 37° and 20° C. (room temperature) showed marked deterioration in six months. Vaccines kept at 15° C. and lower did not show any appreciable reduction in agglutinin-producing properties for fifteen to eighteen months. After twenty-four months, however, a noticeable deterioration had taken place and this was still more evident after storage for thirty months.

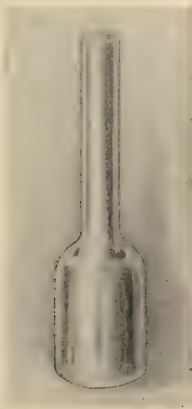


FIG. 85.—A SMALL VACCINE AMPULE.
Capacity 1 c.c.

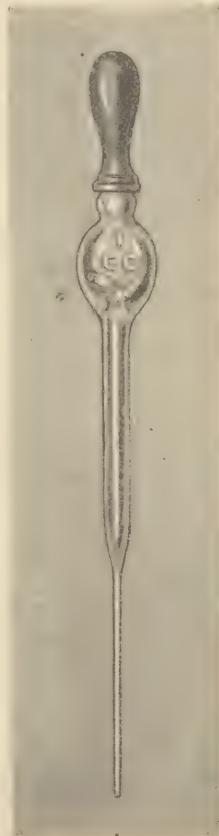


FIG. 86.—COMER'S AUTOMATIC PIPET. (Steele Glass Co., Philadelphia.)

The inner tube to the tip of the pipet holds exactly 1 c.c. If the rubber teat raises too much fluid, the excess is received in the glass reservoir; when too much fluid accumulates in this, it may be emptied by turning the point of the inner tube downward and ejecting the fluid by pressure on the teat.

It would appear, therefore, that ordinary saline vaccines should be kept as far as possible at a temperature below 15° C. and used within six to twelve months. According to some investigations vaccines preserved in glycerol undergo much less deterioration.

PREPARATION OF SENSITIZED BACTERIAL VACCINES

A highly immune serum is prepared by immunizing a series of rabbits or a goat with the micro-organism to be used in preparing the vaccine.

¹ Amer. Jour. Pub. Health, 1913, 3.

² Hygienic Laboratory Bull., No. 122.

The first injections consist of heat-killed emulsions, administered subcutaneously. After the first or second dose the period of heating is gradually reduced, and the dose increased, until finally the injections may be given intravenously and with living micro-organisms. From time to time a small amount of serum should be examined for immune bodies: with the typhoid-cholera group, by testing for bacteriolysin and agglutinins; with staphylococci and streptococci, by agglutination, bacteriotropic, and complement-fixation tests; with pneumococci, gonococci, and meningococci, by bacteriolytic, agglutination, and bacteriotropic tests. When a highly immune serum is secured the animal is bled, the serum isolated, heated to 56° C. for half an hour, and stored in a strictly aseptic manner.

To *sensitize* the bacteria, thick, even emulsions of young cultures in normal salt solution are treated with one-half to an equal bulk of inactivated immune serum, and the mixture gently agitated at room temperature for from six to twelve hours. The emulsion is then thoroughly centrifuged, and the residue of bacteria washed three times with sterile salt solution, after the manner in which the red corpuscles are washed. After the final washing the bacteria are resuspended in salt solution, shaken for a time to insure breaking up of agglutinated clumps, counted, heated at 60° C. for an hour, cultured as a test for sterility, and then diluted so that the emulsion will contain slightly larger doses than a corresponding dose of ordinary vaccine prepared for administration.

Sensitization probably consists in the union of bacteriolytic amboceptor with its antigen, and when injected serves, with the patient's complement, to hasten solution or lysis of the bacteria (antigen), thereby liberating quickly the chemical substances required for the stimulation of antibodies.

Metchnikoff and Besredka are using sensitized *living* bacteria, and their work is being followed with much interest. In this country strict legal restrictions and regulations exist regarding the sending of living cultures through the mails. If, therefore, the method should fulfil the high claims and expectations made for it, there may be considerable difficulty in bringing it into general use.

PREPARATION OF LIPOVACCINES

These vaccines contain the bacterium suspended in oil instead of water or saline solution. They were first mentioned by Warden¹ and later advocated by LeMoignic and Pinoy,² and Whitmore, Fennel and Petersen³ for prophylaxis against typhoid fever. Their chief advantage consists in the slow absorption of the oil permitting the administration of a single large dose of bacteria instead of the three doses of saline vaccine. Antibody production results from the administration of these lipovaccines, but, as shown by Bengston⁴ and others, not as promptly and scarcely to the same degree as follows the administration of equal numbers of bacteria in saline suspension. Whitmore has prepared these vaccines of typhoid and paratyphoid bacilli, pneumococci, meningococci, and other bacteria for prophylactic immunization in the army; technical difficulties, however, and especially sterilization, together with rather severe reactions, have limited the usefulness of lipovaccines, so that they have been largely abandoned in favor of the saline vaccines.

¹ Jour. Amer. Med. Assoc., 1915, 65, 2080.

² Compt. rend. Soc. de biol., 1916, 79, 209.

³ Jour. Amer. Med. Assoc., 1918, 70, 427; *ibid.*, 902.

⁴ Hygienic Laboratory Bull., No. 122.

Whitmore and Fennel have prepared these vaccines as follows:

1. The bacteria are grown on agar in Kolle flasks and removed with a vacuum scraper. Or the bacterium (as the pneumococcus) may be grown in dextrose broth and secured by centrifuging.

2. The bacterial mass is poured in sterile Petri dishes, dried in an oven at 53° C. or frozen and dried in vacuo, a flaky mass resulting which crumbles into a fine powder ready for incorporation into a sterile oil.

3. The bacterial mass is now weighed under sterile precautions and ground in a ball mill for at least forty-eight hours. A few cubic centimeters of chloroform-ether may be added to the mass at the beginning to insure final sterilization.

4. Lanolin sterilized by autoclaving at 15 pounds for thirty minutes is warmed and sufficient added to make 10 per cent. in the completed vaccine; this mixture is ground for another twenty-four hours, when sterile olive or cottonseed oils (autoclaved) are added and grinding continued for twenty-four hours.

5. The final oil suspension is now heated at 53° C. for one hour on a water-bath and cultured for sterility. Lewis and Dodge¹ advise heating the pneumococcus vaccine to 130° C. for three hours which does not appear to injure the immunizing properties, although heating typhoid lipovaccine in this manner greatly injured their immunizing properties.

Pneumococcus lipovaccine has been so prepared that each cubic centimeter contained the equivalent of about 6,000,000,000 of each of the three fixed types (I, II, and III). Meningococcus vaccine has been prepared containing 10,000,000,000 of each type in each cubic centimeter. Dysentery vaccine has been prepared so that each cubic centimeter contained about 2,000,000,000 of each of the three principal types (Shiga, Flexner, and Y types). Typhoid-paratyphoid vaccine usually contained about 2,500,000,000 of each of the three strains of bacilli. These examples serve to show the approximate strength of the various lipovaccines.

Rosenow and Osterberg² have described a method for preparing lipovaccines in which the germs are suspended in water, sterilized with 2 per cent. cresol for twelve hours plus heating to 60° C. for one-half hour, followed by the addition of sterile lanolin and olive oil. The water is then removed by vacuum distillation at about 65° C. and the resulting suspension in oil so diluted with sterile oil that 50,000,000,000 of bacteria are contained in 1 c.c. The authors have also given the following method for the preparation of small amounts of lipovaccines and particularly autogenous vaccines:

"The common 6-ounce nursing bottle has been found useful for the preparation of autogenous lipovaccines. It serves admirably as a culture flask, centrifuge tube, and vacuum flask. The bacteria are grown in tall columns of glucose broth (150 c.c. per bottle) for twenty-four hours, centrifugalized, the supernatant clear broth decanted, and the sediment suspended in 10 c.c. of a 1.5 per cent. solution of purified cresol in water or salt solution. This is thoroughly mixed and placed at 36° C. for from two to fifteen hours, when cultures are made. Streptococci and pneumococci are usually killed in from two to twenty-four hours. As soon as the suspension is found to be sterile it is centrifugalized; the supernatant fluid is decanted, and 6 c.c. of cottonseed oil containing 2 per cent. anhydrous lanolin and a number of sterile glass beads or steel shot are added. The mixture is emulsified by being shaken for a short time. The small amount of water from this water-

¹ Jour. Exper. Med., 1920, 31, 169.

² Jour. Amer. Med. Assoc., 1919, 73, 87.

bacterial-oil suspension is now removed by applying the vacuum and immersing the bottom of the bottle in water heated to 60° C. By means of vigorous shaking at intervals the removal of the water is hastened. The vacuum and the heat are applied until bubbling ceases and the mixture becomes clear. The time required depends on the completeness of the vacuum and the amount of water to be removed, but the clearing usually takes place in from twenty minutes to one hour. If larger amounts of bacteria are required the water or salt solution suspensions of a number of bottles are placed in one, and a correspondingly larger amount of oil is added. By the use of Y-tubes the water from a series of suspensions may be removed at one time.

"If, for example, bacteria such as influenza bacilli, gonococci, and meningococci, which grow better on solid mediums, are to be used, the growth should be scraped together and washed off with salt solution so that the final suspension is roughly equivalent to that containing the bacteria from the broth culture. Sterilization and the further steps are carried out as above. In the case of the more resistant bacteria, such as staphylococci and paratyphoid bacilli, heating the cresolized suspension to 60° C. for one hour hastens the sterilization. The final bacterial content of the lipovaccine is calculated on the basis of counts made of the bacteria suspended in salt solution or on the basis of the total number of bacteria per cubic centimeter of broth culture. In the broth used in our laboratories the amount of growth of pneumococci and streptococci is usually about 2,000,000,000, and the vaccine is made to contain approximately 50,000,000,000 of these organisms per cubic centimeter. The number of bacteria in the oil may be increased ten or twenty fold without interfering materially with the evaporation of the water or with the even distribution of the bacteria."

CHAPTER XIII

ANTITOXINS

For general purposes the antibodies produced during infection may be divided into two groups, the first consisting of those antibodies that are truly antagonistic to the bacterium or its products responsible for their production, and the second those that are not in themselves destructive, but that probably prepare the bacterium for the action of a more powerful antibody of the first group.

To the first group belong the antitoxins, which neutralize the toxins of a bacterium without being directly destructive to the micro-organism itself; and the bacteriolysins, which are truly destructive, causing the bacterium to break up and finally disappear.

To the second group belong the opsonins, which, as we have seen, prepare the bacterium for phagocytosis; and the agglutinins and precipitins, which, while not in themselves destructive, probably in some manner prepare their antigen for the action of bacteriolysins, just as opsonins prepare them for phagocytosis.

Definition.—*Antitoxins are antibodies in the blood that are capable of directly and specifically neutralizing the dissolved toxins that caused their production.*

Historic.—Bacteriolysins were discovered before antitoxins. Their discovery is due to the researches of Nuttall, Fodor, Buchner, and others, who showed that normal serum, and especially the serum of animals artificially immunized against a certain bacterium, was able to exert a destructive action on the micro-organism, causing its dissolution and final disappearance. This property of the blood-serum was found to diminish with age, and to disappear completely when the serum was heated to 56° C. Buchner laid greatest stress upon the importance of the thermolabile substance which he called alexin, but later researches have shown that the main factors are the specific bacteriolysins, which, however, are practically powerless to destroy their antigen without the co-operation of alexin (later renamed "complement" by Ehrlich).

While these studies were being made, in the hope of thus explaining all phases of immunity, Behring discovered that in diphtheria infections induced experimentally, while the animals became more and more immune, virulent bacilli may, nevertheless, be present at the site of injection. Here, then, was an example of immunity that could not be explained on the basis of bacteriolysis. Later, in 1890 and 1892, Behring,¹ in collaboration with Kitasato and Wernicke, made further important discoveries, showing that the blood-serum of animals actively immunized against diphtheria and tetanus would protect normal animals against these diseases, and, furthermore, that the blood-serum of the immune animals did not possess bactericidal properties. These observers also demonstrated that such serum could be used therapeutically for the cure of an infection already in progress.

Soon after these discoveries Ehrlich² showed that specific antitoxins (antiricin, antiabrin, etc.) could also be produced against the poisons of some plants, and Thisalix and Bertrand,³ and Calmette⁴ produced a similar

¹ Deutsch. med. Wchn., 1890, 1145, 1245.

³ Compt. rend. Soc. de biol., 1894, 111.

² Ibid., 1891, 976, 1218.

⁴ Ibid., 1894, 120, 204.

antitoxin (antivenin) against snake poison. Other observers since then have increased the list of poisons against which antitoxins can be produced; as, for example, Kempner has produced an antitoxin against the poison of *Bacillus botulinus*, and Wassermann one against that of *B. pyocyaneus*.

Formation of Antitoxins.—It was formerly believed that there was a direct conversion of toxin into antitoxin, but this certainly is not the case, for the amount of antitoxin produced is altogether out of proportion to the amount of toxin injected.

Antitoxins are formed by those cells that anchor the toxins. In order to produce them it is necessary that the toxin enter into direct union with the cells and exert a stimulating influence on them, for where a loose union occurs, as between cells and alkaloids, antibodies are not formed.

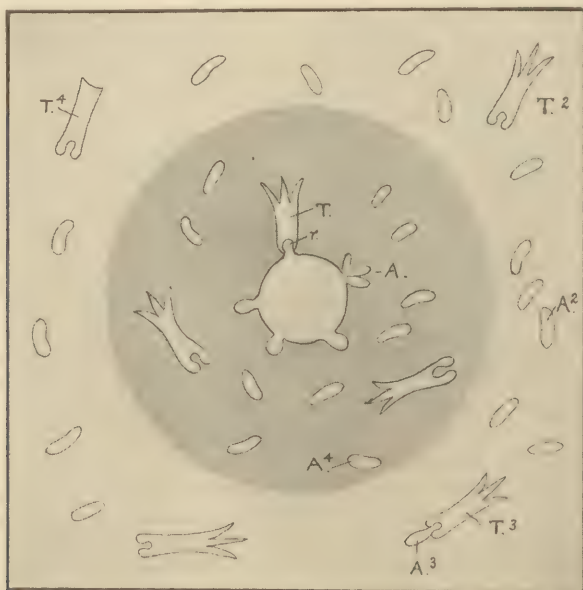


FIG. 87.—THEORETIC FORMATION OF ANTITOXINS.

The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell.

r, A receptor of the molecule (first order); *A*, overproduction of receptors, which are being cast off; *A*², a cast-off receptor free in the body fluids—now an antitoxin; *A*³, a molecule of antitoxin combination with a toxic molecule *T*³; *A*⁴, a cast-off receptor still within the parent cell; *T*, a toxin molecule in combination with the receptor of a cell molecule; *T*², a toxin molecule free in the body fluids; *T*³, a toxin molecule in combination with antitoxin; *T*⁴, a molecule of toxoid (toxophore group lost).

Having entered into chemical union with the side arms of cells, a toxin may destroy the entire cell, and if a sufficient number of these are destroyed, the host will show symptoms of infection and may succumb. If, however, the cell itself is not destroyed, but only one or more of the side arms injured, the damage is repaired by the cell forming new side arms that have a specific affinity for the toxin responsible for their production. According to Weigert's overproduction theory, a cell once stimulated to produce these side arms or receptors continues to produce them for some time, even after the stimulus has been removed. In this manner the specific receptors are produced in excess, and since all cannot remain attached to the parent cell, the excess is discharged into the blood-stream. Each of these cast-off receptors is capable of uniting with toxin, thus neutralizing the poisonous

principles of the toxin and rendering it practically harmless. *Antitoxins, therefore, are nothing more than these cast-off receptors, which have a specific affinity for their toxins* (Fig. 87).

As Adami has pointed out, it is probable that the toxins exist for some time within the cell, not as part and parcel of the cell, but as a stimulating agent that causes the cell to develop the habit of producing the specific receptors. The mere union of toxin with a receptor, causing it to fall off, and being followed by nature's mode of repair, with the formation of an excess of receptors and no further stimulation, is hardly sufficient to explain the enormous activity of the cells.

That antitoxins may be produced locally was illustrated by the experiment of Romer with abrin. This substance has a peculiarly powerful effect upon the conjunctiva. By gradually immunizing the right conjunctiva of a rabbit with increasing doses, it was shown that, after killing the animal and triturating the conjunctiva with a fatal dose of abrin, an injection of the emulsion of the right or immunized conjunctiva was without effect, whereas the emulsion from the left proved fatal. Thus it will clearly be seen that the cells that had absorbed the abrin had developed and contained antiabrin in sufficient amounts to neutralize the poison.

A single attack of diphtheria does not cause the production of large amounts of antitoxin. For this, repeated, possibly minute infections are more effectual, which pass over with minimal or misinterpreted symptoms. Otto¹ found that diphtheria carriers, both those who had had the disease and those who had not, contained more antitoxin in their blood than patients who had just recovered from an attack. This indicates that the mere presence of bacilli in the throat is sufficient to stimulate the production of antitoxin on which the immunity of the carrier himself would seem to depend.

While leukocytes, such as Metchnikoff's macrophages, are likewise active in the formation of antitoxins, it is certain that they are not the only cells involved. Metchnikoff claims that antitoxins are merely toxins altered by leukocytic activity, rather than constituents of tissue cells; this explanation is, however, inadequate, and it has been shown experimentally that the quantity of antitoxin produced is so far in excess of the amount of toxin injected as to render this view untenable.

Structure of Antitoxins.—According to the side-chain theory, antitoxins are the simplest of antibodies, being composed of a single arm or haptophore group for union with the toxin, and called *receptors of the first order*. While illustrations of this theoretic structure will convey the impression of mere physical contact or union with toxin, it is to be remembered that experimental data indicate that the union and consequent neutralization of the toxin are chemical processes.

Properties of Antitoxins.—While chemical analyses to determine the nature of antitoxin serums were made as early as 1897, little is known regarding it because it is impossible to secure the antitoxic element free from serum and serum constituents. Brodie² was among the first to show that diphtheria antitoxin was completely precipitated from a solution by any means which removed the globulins, and his observations were extended by Belfanti and Carbone,³ and by Seng.⁴ But the first notable advance in this study was made by Hiss and Atkinson,⁵ who estimated the globulin

¹ Deutsch. med. Wchn., 1914, March 12, 542.

² Jour. Path. and Bacteriol., 1896, 4, 460.

³ Abstr. Centralbl. f. Bakteriolog., 1, 1898, 23, 906.

⁴ Ztschr. f. Hyg. u. Infektionskrankh., 1899, 31, 513.

⁵ Jour. Exper. Med., 1900, 5, 47.

content of the serum of a large number of horses at different stages of immunization against diphtheria toxin. As a result of these experiments these workers arrived at the conservative conclusion that a low potency coincided with a low globulin content, but that it was not possible to regard the absolute amount of globulin as an index of the antitoxin content of the serum. Similar studies were made by Ledingham¹ and by Gibson and by Banzhaf.² The former concludes from the data furnished by the immunization of a horse and a goat with diphtheria toxin that there would seem to be "some intimate relation between the amount of antitoxin developed and the quantity of the globulins." Gibson and Banzhaf are still more guarded in their conclusions. These workers state that while the greatest rise in the serum globulin was usually coincident with maximum antitoxic potency, the extent of this increase was practically independent of the antitoxin potency when the results on more than one horse were contrasted. They, therefore, are inclined to the view that there may be no relation between the absolute or percentage increase of the serum globulin and the antitoxic potency in the plasma of different horses; indeed, they have shown that the increase in the serum globulin of refractory horses may surpass that in the plasma of some of those yielding a high antitoxin. Similar deductions were made by Banzhaf and Famulener³ in the immunization of goats. They found that the total protein content and the protein partition may be normal at a time when the animal shows the maximum number of antitoxic units. Meyer, Hurwitz, and Taussig^{4,5} subscribe to the view that the globulin change is not a necessary concomitant of the elaboration of antibodies, although it is now well established that this protein fraction may increase strikingly during the process of immunization. Their conclusions have been derived from parallel studies of the serum protein fractions and of the antitoxin content of a number of different animals immunized not only with diphtheria toxin but also with the soluble toxins of the bacilli of tetanus and of botulism. In general, the results obtained in the different animals and with the different soluble toxins point in the same direction and lend support to the view that in animals immunized with bacteria and their toxins the curves of serum globulin increase and the development of antitoxin potency do not run parallel. Gibson and Banzhaf showed that the portions of the globulin precipitate soluble in saturated sodium chlorid solution carried most of the antitoxin, and with this discovery a practical method of eliminating much of the non-antitoxic portion of the serum was perfected.

The relation of antitoxins to proteins has also been studied, digestive ferments being permitted to act on antitoxic serum. It has been shown that antitoxin resists tryptic digestion to a well-marked degree; in this respect it resembles the serum globulin. All the evidence obtained indicates that a closer relation of antitoxins to proteins exists than has been shown for the toxins, although all attempts to separate antitoxins from proteins have thus far failed.

At the Bureau of Animal Industry in Washington, Berg and Kelser⁶ have attempted anew to secure a protein-free antitoxin preparation on the one hand, and, on the other, to determine whether antitoxin can be destroyed by procedures that leave the protein intact. The outcome of these

¹ Jour. Hyg., 1907, 7, 65.

² Jour. Exper. Med., 1910, 12, 411.

³ Coll. Stud. Research Lab., Dept. Health, New York, 1915, 8, 208.

⁴ Jour. Exper. Med., 1916, 24, 515.

⁵ Jour. Infect. Dis., 1918, 22, 1.

⁶ Proc. Nat. Acad. Sci., 1918, 4, 174.

experiments was to show that antitoxin destruction may take place with or without cleavage of protein and the authors suspected that tetanus antitoxin, for example, may be a substance of non-protein nature. The evidence, however, is not conclusive so long as a protein-free antitoxin is not obtained. Of great interest in this connection are the investigations of Huntoon, Masucci and Hannum,¹ who have succeeded in preparing solutions carrying antipneumococcus antibodies which give but feeble chemical reactions for the proteins and apparently fail to sensitize guinea-pigs.

Antitoxins are fairly resistant bodies, and a properly prepared antitoxic serum, when kept in a cool place and protected from light and air, may be preserved for a year or more with very little deterioration in strength. At times, however, for unknown reasons antitoxins gradually deteriorate, losing about 2 per cent. in strength a month. Anderson² has calculated the yearly loss in potency at about 20 per cent., although occasionally it may go as high as 25 per cent. when the serum is kept at room temperature. At 15° C. the yearly loss was about 10 per cent. and at 5° C. about 6 per cent. Old sera, unit for unit, were found just as potent as fresh sera. But little difference was noted in the keeping qualities of whole serum and solutions of the globulins. Manufacturers have endeavored to calculate this loss in strength, and have placed a label on each package of antitoxin, bearing a date beyond which the serum is not guaranteed to contain the amount of antitoxin present at the time it was put up.

The antitoxins, with few exceptions, are far more stable than the toxins, resisting heating up to 62° C., but gradually deteriorating with higher temperatures. Boiling destroys them completely. They are readily preserved with small amounts of chloroform, phenol, tricrosol, etc., although strong solutions of these produce destructive changes. Putrefaction of the serum destroys the antitoxin content. Ehrlich has devised the best method for their preservation, which consists in drying the serum *in vacuo* and preserving it in the dark, at a low temperature, in the presence of anhydrous phosphoric acid. So preserved, antitoxin retains its strength for prolonged periods and is used in standardizing toxins.

Natural Antitoxins.—The appearance of so-called natural antitoxins can be explained on the basis of Ehrlich's theory. Since the antitoxin is composed of receptors that are not new bodies, but simply normal receptors produced in excess, it is reasonable to assume that a few may be thrown off occasionally, constituting the natural antitoxin.

Small amounts of natural diphtheria antitoxin may be found in certain individuals. Since the diphtheria bacillus is so wide-spread in its distribution, it is possible that minor subinfections may be responsible for antitoxin production, and this is probably always the case when large amounts are found.

There are numerous examples of natural antitoxin immunity among the lower animals, the most notable being the resistance of the rat to diphtheria toxin and the chicken to tetanus toxin. The investigations of Burrows and Suzuki³ and Suzuki,⁴ employing cultures of tissues, have indicated that this resistance is due to antitoxins in the plasma and to special resistance of certain cells of these animals.

Information regarding natural antitoxins for other members of the toxin-producing group of micro-organisms is less complete, although it is highly probable that natural antitoxins for these exist.

¹ Jour. Immunology, 1921, 6, 185.

² Jour. Infect. Dis., 1910, 7, 481.

³ Jour. Immunology, 1918, 3, 219.

⁴ Jour. Immunology, 1918, 3, 233.

The Schick Test for Natural Diphtheria Antitoxin.—Schick¹ has worked out a simple and practical skin test which apparently has proved satisfactory and trustworthy and of distinct value for detecting natural immunity to diphtheria among human beings.

This test consists in the *intradermic* injection of a minute dose of diphtheria toxin. If the individual possesses an amount of antitoxin equal to at least one-thirtieth of a unit in each cubic centimeter of blood-serum the injected toxin is neutralized and no reaction follows; if, however, the individual does not have antitoxin in the body fluids the injected toxin acts as an irritant to the skin, producing in twenty-four to forty-eight hours a small area of redness and edema. A positive reaction indicates that the individual does not possess natural antitoxin in his blood and, therefore, that he is susceptible to diphtheria; a negative reaction indicates that natural antitoxin is present and that he is, in all probability, immune to diphtheria. In the presence of exposure to diphtheria persons reacting positively to the toxin skin test should receive a prophylactic dose of antitoxin, while those reacting negatively may with safety be spared the injection. A detailed description of this test is given in a later chapter.

Specificity of Antitoxins.—Antitoxins well illustrate the law of specificity that exists between antigen and antibody, since they are strictly specific for their toxins. Diphtheria antitoxin will neutralize only diphtheria toxin; tetanus antitoxin, only tetanus toxin, and so on through the list. This specificity is not confined to the particular toxin-producing organism that generates the antitoxin; for example, there are various kinds of diphtheria bacilli, differing as regards morphology and toxicity, although one antitoxin appears to act the same with their various toxins.

Types of Toxic-producing Bacilli; Monovalent vs. Polyvalent Antitoxic Sera.—Of considerable interest and practical importance is the question whether different types of diphtheria, tetanus, and other toxin-producing bacilli produce toxins corresponding to type and whether these are neutralizable by monovalent antitoxins for each of the respective species of bacilli.

Several types of tetanus bacilli are recognized according to their response to agglutinins, but the toxins of all are neutralized by a monovalent antitoxin. Durand² and Havens³ have found different types of diphtheria bacilli on the basis of agglutination tests, but the toxins of all of these are neutralized by a monovalent (No. 8) antitoxin according to the studies of Park, Williams, and Mann.⁴ Practical experience with tetanus and diphtheria antitoxins in the prophylaxis and treatment of tetanus and diphtheria has abundantly proved the efficacy of the monovalent antitoxins for these two diseases as discussed in Chapter XL. However, *Bacillus botulinus* shows at least two different types producing toxins which respond only to homologous antitoxins.

Nature of the Toxin-antitoxin Reaction.—While the injection of toxin-antitoxin mixtures into the lower animals is the only practical method of testing and standardizing the curative and prophylactic powers of their serums, this method does not throw much light upon the nature of the toxin-antitoxin reaction, or show how antitoxin overcomes the toxin.

Antitoxin is protective and curative, in that it actually destroys the toxin in a manner similar to the dissolution of a bacterium caused by a

¹ Münch. med. Woch., 1913, lx, 2608.

² Jour. Infect. Dis., 1920, 26, 388.

³ Compt. rend. Soc. Biol., 1918, 81, 1011 and 1920; *ibid.*, 1919, 83, 611 and 613.

⁴ Jour. Immunology, 1922, 7, 243.

specific bacteriolysin; or it may influence the tissue cells in some way and render them more resistant to the toxins, a view that was held by Roux, and particularly by Buchner; or the antitoxin may form a direct chemical union with the toxin, similar to the chemical neutralization of an acid by a base—an opinion early held by Behring and elaborated later by Ehrlich.

Experimental data support the view of chemical union with the toxin. In the test-tube some time is required for the union of toxin and antitoxin to occur; this union is hastened by heat and retarded by cold; it is more rapid in concentrated than in dilute solutions, and in general takes place in accordance with the law of multiple proportions—all of which tends to show the close similarity of the toxin-antitoxin reaction to a chemical process.

It is generally conceded that antitoxin does not directly destroy the toxin, for when neutral mixtures of toxin and antitoxin are injected into animals, portions of toxin may become dissociated and unite with tissue cells possessing greater affinity for the toxin, and symptoms of infection may result. It is probable that toxin and antitoxin form a distinct compound, and this action requires time for its consummation. For example, Martin and Cherry, by filtering mixtures of toxin and antitoxin through fine filters that would permit the toxin molecule to pass through but restrain the larger antitoxin molecule, found that, if filtered immediately, all the toxin in the mixtures was extruded, but that, as the interval between mixing and filtration was prolonged, less and less toxin appeared in the filtrate, until finally, two hours after mixing, no toxin whatever passed through the filter.

This element of time in support of the chemical nature of the reaction is further strengthened by the experiments of Calmette with snake venom and antivenin, and likewise serves to demonstrate that the antitoxin apparently does not directly destroy the toxin. Although most toxins are thermolabile, Calmette found that snake venom is rendered inert by heating to 68° C., whereas the antivenin remains uninfluenced by a temperature of 80° C. When neutral mixtures of venom-antivenin were heated to 70° C., they were found to become toxic again, presumably on account of the destruction of the antivenin, the venom itself not being destroyed. Similar experiments were carried out by Wassermann with mixtures of pyocyanous toxin-antitoxin, with similar results. In both instances, however, as developed later, if the mixtures had been allowed to stand longer, these results would not have been secured. Although performed originally to show that an antitoxin does not act by actually destroying its toxin, these experiments simply demonstrate the importance of the element of time in the reaction, without throwing any real light upon the nature of the new toxin-antitoxin compound, if such exists.

That toxin is counteracted by antitoxin, independent of the participation of living tissue cells, has been quite conclusively proved by experiments *in vitro*. Ehrlich showed that the agglutinating qualities of ricin—a vegetable toxin—may be overcome in the test-tube by adding antiricin, the corresponding antitoxin. Similar results were obtained by Ehrlich with tetanolysin and tetanus antitoxin, and by Stephens and Myers with cobra venom and its antivenin.

It is probable that antitoxin has a similar action when injected for therapeutic purposes, as for curing an infection. The longer the interval that has elapsed between the time of infection and the administration of antitoxin the less satisfactory will be the result, as antitoxin becomes less powerful when toxins have formed a firm union with the body cells. This

is especially true in tetanus, where even very large doses of antitoxin may be incapable of dissociating the toxin molecule from the nerve cells, the serum, therefore, being of greatest value in prophylaxis. In diphtheria, however, the union between toxin and cells is less firm, and the antitoxin is probably capable of neutralizing the toxin already present in the cells, and especially any toxin that may become dissociated from the cell or is freshly prepared by the diphtheria bacillus at the site of infection. The indication, therefore, in giving antitoxin, is to give a dose large enough to neutralize all free and loosely bound toxin, with an excess to neutralize dissociated toxin and that prepared by the bacillus during the course of the infection.

The introduction of the test-tube experiment into the investigation of these reactions permitted more exact observations to be made, and the evidence secured by this means, as well as by carefully graded quantitative animal experiments, would seem to indicate that we should accept, for the present at least, the conception of the chemical nature of the process.

PRODUCTION OF ANTITOXINS FOR THERAPEUTIC PURPOSES

Diphtheria and tetanus antitoxins are manufactured on a large scale and are used extensively in the prevention and cure of these infections. They are prepared by immunizing horses with carefully graded and increasing doses of the respective toxins until the serum of the animals shows a sufficiently high antitoxin content, after preliminary trials, to warrant more extensive bleeding. Large quantities of blood are then collected aseptically by puncturing the jugular vein. The serum is carefully separated and standardized according to an accepted technic in order to determine the antitoxin content in units. A small amount of preservative is added, and the serum is finally dispensed in special containers or syringes ready for administration. In some laboratories it is customary to precipitate the globulin fraction of the serum with magnesium or ammonium sulphate, and redissolve the portion containing most of the antitoxin in saturated sodium chlorid solution. The bulk of the serum is thus greatly decreased and objectionable constituents largely eliminated, to the obvious advantage of the preparation for therapeutic purposes.

Antitoxins have also been prepared for other bacterial toxins, as those of the dysentery bacillus (Kruse-Shiga) and *Bacillus botulinus*, for the vegetable toxins in pollen and for the animal toxins in snake venoms.

There are other serums for the treatment of certain infections, which depend for their effects chiefly upon the presence of bacteriolysins and immune opsonins, and these are described in a subsequent chapter.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN

The following, taken largely from Park,¹ is a widely used and accepted technic for the production of *diphtheria antitoxin*:

Production of the Diphtheria Toxin.—A strong diphtheria toxin should be obtained by growing a virulent culture in a 2 per cent. nutrient peptone bouillon made from "bob" veal, of an alkalinity that should be about 9 c.c. of normal soda solution per liter above the neutral point to litmus, and prepared from a suitable peptone (Witte). The broth should be poured into large-necked Erlenmeyer flasks in comparatively shallow layers so as to allow of the free access of air, and maintained at a temperature of about 35° to 36° C. (Fig. 88).

¹ Park and Williams, *Pathogenic Micro-organisms*, Lea & Febiger, 1920, 181.

In the Hygienic Laboratory of the Public Health and Marine Hospital Service "Smith's bouillon" is used for preparing the toxin. This is made of fresh lean beef, after the muscle sugar and all other sugars have been removed by fermentation with a good culture of *Bacillus coli*. The reaction is adjusted until 0.5 per cent. acid to phenolphthalein, that is, still distinctly alkaline to litmus, and 1 per cent. peptone, 0.5 per cent. sodium chlorid, and 0.1 per cent. dextrose are added. The reaction is again noted and adjusted to + 0.5 per cent. The broth is then filtered through filter-paper into flasks and test-tubes and sterilized in the autoclave at a temperature of 120° C. for twenty minutes.

After incubating for from seven to ten days the culture is removed, and its purity having been tested by microscopic and cultural methods, it is rendered sterile by the addition of 10 per cent. of a 5 per cent. solution of carbolic acid. After forty-eight hours the dead bacilli have settled on

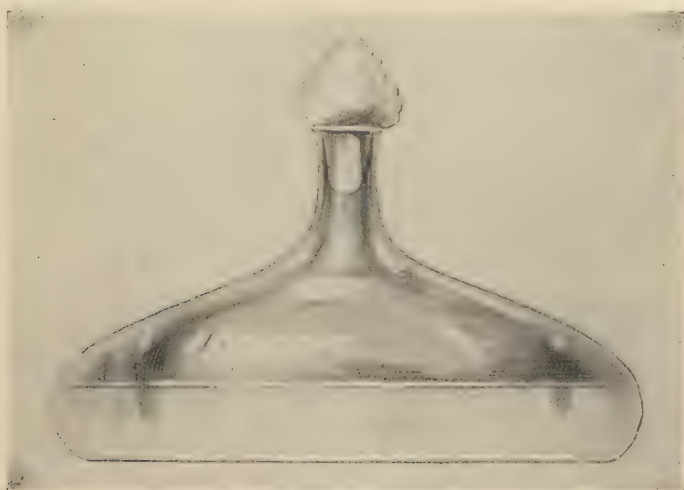


FIG. 88.—A FLASK OF DIPHTHERIA CULTURE.

The bacilli grow on the surface and form a scum. As the culture grows older the bacilli die and sink to the bottom of the flask. A flask of this shape affords a large surface of culture-medium in contact with oxygen and facilitates toxin production.

the bottom of the jar, and the clear fluid above is siphoned off, filtered, and stored in full bottles in a cold place until needed (Fig. 89).

The relation of reaction (alkalinity) of the broth has been studied by Hitchens¹ and many others. Davis² has recently advocated the use of a bouillon with an initial reaction falling within a certain zone of alkalinity varying in hydrogen-ion concentration from 7.0×10^{-8} to about 5.0×10^{-9} . He has found that diphtheria bacilli cultivated in plain broth produces an initial increase in hydrogen-ion concentration, followed by a steady decrease until a limited alkalinity is obtained. No direct relationship was found between hydrogen-ion concentration of the medium and toxicity. Davis³ has cautioned against the presence of any fat in the broth, as it interferes with pellicle formation, and found beef infusion broth with 2 per cent. peptone and 0.5 per cent. salt to be most satisfactory ($\text{pH} = 8.2$). Large

¹ Jour. Med. Research, 1904, 13, 523.

² Jour. Lab. and Clin. Med., 1917, 3, 358.

³ Jour. Bacteriology, 1920, 5, 477.

flasks of this broth inoculated with twenty-four-hour cultures in "starter flasks" cultivated at 36° to 38° C. for ten to twelve days was found to give the largest yields of toxin.

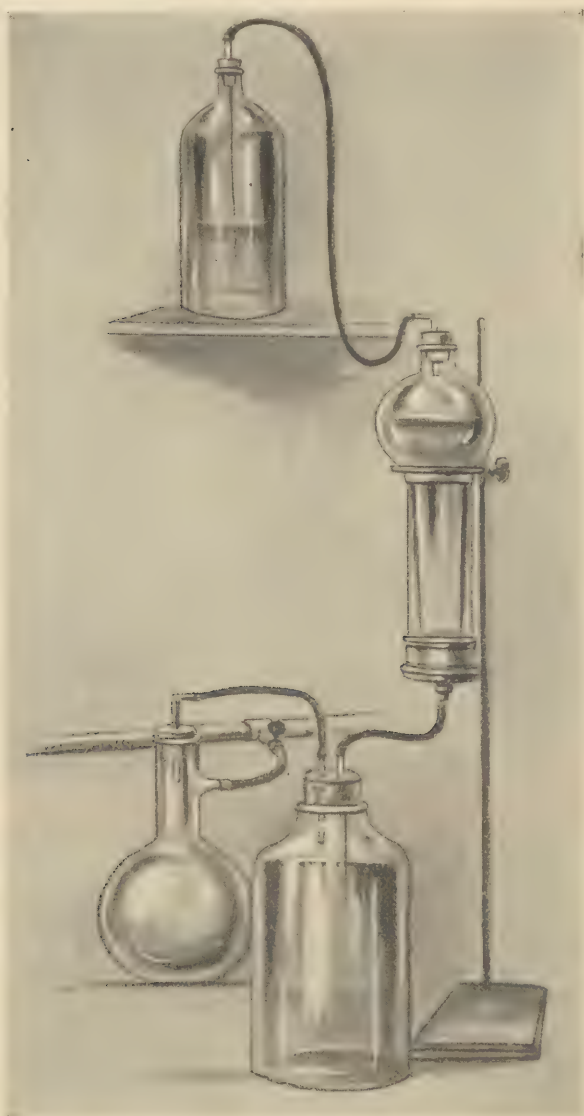


FIG. 89.—A LARGE TOXIN FILTER.

The culture is contained in the large bottle on the shelf and drains into the flask, which, in turn, empties into the earthen "candle." By means of a vacuum the culture is filtered through the "candle" and collects in the large bottle at the base of the stand.

As shown by Robinson and Rettger¹ diphtheria toxin cannot be produced unless complex nitrogenous bodies are present, as some of the proteoses; in protein-free media there is very little toxin production. During

¹ Jour. Med. Research, 1917, 36, 357.

recent years considerable trouble was occasioned by lack of sufficient quantities of Witte's peptone, but American brands of peptone are now yielding satisfactory results.

Testing the Toxin.—The strength of the toxin is then tested by injecting a series of guinea-pigs with carefully measured amounts. When injected hypodermically, less than 0.005 c.c. should kill a 250-gram guinea-pig, and a toxin requiring more than 0.01 c.c. to kill a pig of this weight is too weak for present purposes. This preliminary titration of the toxin will suffice for determining the dosage for horses, but in standardizing antitoxin the technic must necessarily be more accurate.

Immunizing the Animals.—The horses used should be young, vigorous, of fair size, and absolutely healthy. They should be severally injected with 3000 units of antitoxin and the following day with 5000 units of toxin, an amount sufficient to kill 5000 guinea-pigs, each weighing 250 grams, or 10 M. L. D. of toxin diluted to 50 c.c. with saline solution. If antitoxin is not given with the first doses of toxin, only one-tenth of the *dose advised is to be given*. After from two to four days, or as soon as the temperature reaction has subsided, a second subcutaneous injection of a larger dose is given, the amount of toxin increasing about 100 per cent. per dose for the first seven to eight injections. The rate of increase is 75 per cent. for the next seven to eight injections, and 50 per cent. for the next series. The rate of increase is then gradually lowered to 10 per cent., which is maintained until the maximum for the horse is reached. At the end of this time a trial bleeding is made and the serum tested.

There is absolutely no way of judging which horses will produce the highest grades of antitoxin. Roughly estimated, those horses that are extremely sensitive and those that react feebly are the poorest, but there are exceptions even in these cases. The only reliable method, therefore, is to bleed the horses at the end of six weeks or two months and test their serum. As shown by Park and Zingher, persons yielding negative Schick tests respond to toxin-antitoxin injections with the production of antitoxin more readily than persons who react in a positive manner. Taking advantage of this data, Hitchens and Tingley¹ have injected 0.2 c.c. diphtheria toxin equal to 3 M. L. D. for 250-gram pigs into the conjunctiva of one eye of horses under examination for the purposes of immunization, and found that many yielded negative tests read at the end of forty-eight hours, indicating the presence of natural diphtheria antitoxin in the blood of normal horses; these animals are probably to be preferred in the production of antitoxin, as they are likely to yield highly potent sera. If only high-grade serum is wanted, all horses that give less than 150 units per cubic centimeter should be discarded. The remaining horses should receive steadily increasing doses, the rapidity of the increase and the interval of time between the doses (three days to one week) depending somewhat on the reaction following the injection, an elevation of temperature of more than 3° F. being undesirable.

For example, according to Park, a horse that yielded an unusually high grade of serum was started on 12 c.c. of toxin ($\frac{1}{100}$ c.c. fatal dose), together with 10,000 units of antitoxin. Sixty days later a dose of 675 c.c. was given, and the serum contained 1000 units of antitoxin per cubic centimeter. Regular bleedings were made weekly for the next four months, at the end of which time the serum had fallen to 500 units in spite of weekly gradually increasing doses of toxin. At the end of three months the antitoxic serum of all the horses should contain over 300 units, and in about 10 per cent.

¹ Jour. Amer. Med. Assoc., 1917, 68, 1660.

as much as 800 units in each cubic centimeter. Not more than 1 per cent. give above 1000 units and, according to Park, so far none has given him as much as 2000 units per cubic centimeter. The very best horses, if pushed to their limit, continue to furnish blood containing the maximum amount of antitoxin for several months and then, in spite of increasing injections of toxin, begin to furnish blood of gradually decreasing strength. If an interval of three months' freedom from inoculation is allowed once every nine months the best horses will furnish high-grade serum for from two to four years.

Kraus and Sordelli¹ have recently described a new method for the preparation of diphtheria and tetanus antitoxins and antivenins, consisting in the use of old horses, and making the injections of toxin neutralized with antitoxin twice a week in progressive doses. These investigators claim to have prepared potent sera in twenty weeks by this method.

Collecting the Serum.—In order to obtain the serum the neck of the horse should be cleansed thoroughly as for an aseptic operation, and a special tourniquet applied to distend the jugular vein. A small slit is made through the skin over the vein and a special sharp-pointed cannula is passed upward under the skin for 2 inches or more, and then plunged into the vein. From 6 to 12 liters of blood are collected by a rubber tube into cylindric jars provided with special tops, facilitating filling with blood and subsequent withdrawal of the serum. The cannula, tubing, jars, and everything used in collecting the blood and serum should be carefully sterilized, and the whole operation should be conducted with scrupulous aseptic care in order to avoid contamination (see Fig. 39).

The jars are set aside (Fig. 90) for three or four days, and the serum is drawn off by means of sterile glass and rubber tubing and stored in large sterile bottles. When the globulins are to be separated the blood may be added directly to one-tenth of its volume of a 10 per cent. solution of sodium citrate, which prevents clotting of the blood. Penfold has recently described a method of bleeding directly into oxalate solution, and after sedimentation of the corpuscles has occurred the supernatant plasma is drawn off and the corpuscles reinjected intravenously into the horse. By this method Penfold states anemia and other bad effects from too frequent bleedings may be avoided, and that as much as 60 liters of high potency blood may be taken from a horse in ten days.

The serum should be clear and free from blood and its sterility should be proved by culture tests. An antiseptic, such as 0.4 per cent. tricesol, 0.5 per cent. phenol, or chloroform, may be added, but this is not necessary unless it is desired to keep the serum for some time. The serum is poured into small bottles fitted with rubber stoppers, or placed in special syringes labeled with the number of units contained. The whole process should be conducted with scrupulous aseptic technic. Diphtheria toxin varies too much to be used as a standard in determining the antitoxin content of a serum; hence, a dried antitoxin is prepared by the Hygienic Laboratory and is distributed for this purpose. The serum is evaporated and dried *in vacuo* by passing dry sterile air heated to 35° C. through it and, when perfectly dry, is preserved in special containers over anhydrous phosphoric acid at a constant temperature of 5° C. Preserved in this manner, the antitoxin is quite stable. Just before use it is dissolved in the required amount of sterile normal salt solution.

Method of Concentrating Serum by Isolating the Antitoxin Globulins.—The use of concentrated serum has lessened the incidence of serum sickness

¹ Prensa Medica, 1918, 4, 307.



FIG. 90.—PREPARATION OF DIPHTHERIA ANTITOXIN. SEPARATION OF BLOOD-SERUM.
The bottle on the left shows blood after standing about an hour; the bottle on the right shows the separation of serum about twelve hours after bleeding.

and facilitates the administration of large doses. The first practical method for the concentration and refinement of diphtheria antitoxin was devised by Gibson.¹ Banzhaf² later developed a somewhat different method which was adopted by the American Public Health Association in 1915 with but one modification, consisting of holding the heated plasma-ammonium sulphate mixture at 60° C. for fifteen minutes before filtering. Briefly, the method of Banzhaf is as follows: "The citrated plasma is diluted with half its volume of water and saturated ammonium sulphate solution is added up to 30 per cent. saturated solution. This mixture is heated up to 60° C. and held there for one hour. Then filtered while hot. The precipitate contains the native non-antitoxic proteins and a large amount of non-antitoxic proteins newly formed by the above method of heating. This precipitate is discarded. The filtrate is brought up to 50 per cent. saturated ammonium sulphate solution. The resulting precipitate contains only pseudoglobulin and antitoxin and is pressed to remove excess of fluid, followed by dialyzation until free from salts. After dialysis is completed 0.8 per cent. sodium chlorid is added for isotonicity and 0.3 per cent. tricresol for preservation. It is then filtered through paper pulp and a Berkefeld clay filter, tested for sterility and potency, and filled into sterile syringes or bottles. This method gives a concentration of about six times the original potency" (Park).

Heineman³ has recently described a process consisting of a combination of well-known methods and persistent repetition of certain details until the desired result is obtained. He states that more of the non-antitoxic proteins are eliminated with a product of higher concentration of antitoxic globulins, and that original plasma containing but 100 to 200 units of antitoxin can be used to advantage.

As shown by Berg⁴ filtration through a Berkefeld type of filter does not remove appreciable amounts of antitoxin.

Standardizing the Serum.—During the earlier investigations it was believed that toxin was quite stable, and that it possessed a definite toxicity with a constant value in neutralizing antitoxin. Upon these suppositions the original Behring-Ehrlich antitoxin unit was based, consisting of 10 times the amount of antitoxin that neutralized 10 fatal doses of toxin. For example, if the minimal lethal dose (M. L. D.) of toxin was 0.001 c.c., and 0.01 c.c. was neutralized by 0.01 c.c. of serum, then 0.1 c.c. of serum equaled one unit, or 10 units in a cubic centimeter. Later, stronger serums were found, and von Behring and Ehrlich modified the unit, which they now call the *immunity unit*, to be that quantity of antitoxin which will neutralize 100 times the minimal fatal dose for a 250-gram guinea-pig.

It was soon discovered that toxins are unstable compounds and that almost immediately after their production they begin to change into toxoids, which are not acutely poisonous, but which retain their power to neutralize antitoxin.

In order to standardize a serum it is necessary that the strength of the toxin be known and, since this is so variable, a standard antitoxin is supplied by the Hygienic Laboratory, by which the various antitoxin plants may measure the strength of their toxins. By mixing varying quantities of toxin with one unit of this standard antitoxin and injecting these into 250-gram guinea-pigs, the L₊ (*limes death*) dose is obtained, which is the dose of toxin required to kill a pig in four days with the one unit of antitoxin.

¹ Jour. Biol. Chem., 1906, 1, 161.

² Collected Studies from Research Laboratories, New York, 1912-1913, 7, 114.

³ Jour. Infect. Dis., 1916, 19, 433.

⁴ Jour. Infect. Dis., 1921, 29, 86.

In order to accurately determine this dose many pigs may be required, but this method of titration is the key-note to successful standardization.

Such a titration, for instance, has shown a toxin to react as follows:

TABLE 1.—METHOD OF DETERMINING THE L_+ DOSE OF DIPHTHERIA TOXIN

One antitoxin unit	+ 0.2	c.c. toxin	= No visible symptoms.
"	"	" + 0.22	" " = No symptoms.
"	"	" + 0.24	" " = Usually no symptoms or a very slight reaction.
"	"	" + 0.25	" " = Very slight congestion and edema.
"	"	" + 0.26	" " = Slight edema at site.
"	"	" + 0.28	" " = Edema; sometimes late paralysis.
"	"	" + 0.3	" " = Acute edema and sometimes death.
"	"	" + 0.32	" " = Always acute death about the fourth day.
"	"	" + 0.34	" " = Death from second to third day.
"	"	" + 0.35	" " = Death about the second day.

Here the L_+ dose is 0.32 c.c. The dose of toxin that just neutralizes the antitoxin without causing symptoms has been called by Ehrlich the *Lô* (*limes zero*) dose, and in this instance it is about 0.24 c.c. This determination, however, has not the same practical value as the L_+ dose.

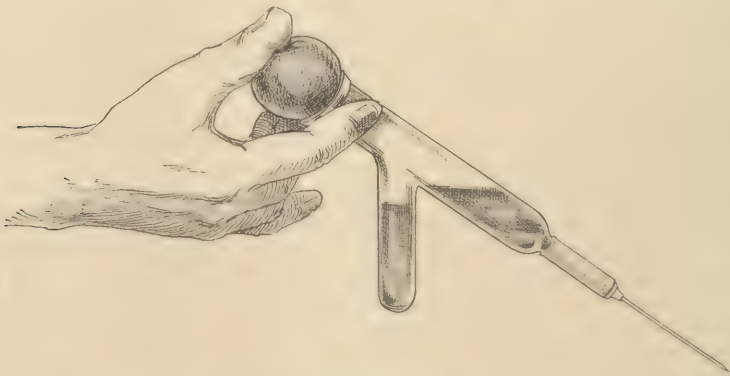


FIG. 91.—A HITCHENS SYRINGE.

The needle is plugged by dipping the tip in carbolized vaselin. The side arm holds sterile salt solution; when the needle has been entered the injection is given by pressure on the bulb; the side arm is then turned upward, and the contents flow into the main barrel; when injected in this manner insures accuracy in dosage and uniform bulk of inoculum.

Having determined the L_+ dose of the toxin a series of six to eight guinea-pigs are injected with this constant dose of toxin and increasing amounts of the corresponding antitoxin serum; for instance, No. 1 would receive 0.001 c.c. of serum; No. 2, 0.002 c.c.; No. 3, 0.003 c.c.; No. 4, 0.004 c.c.; No. 5, 0.005 c.c.; No. 6, 0.006 c.c., etc. If at the end of the fourth day Nos. 1, 2, 3, and 4 were dead, and Nos. 6 and 5 were alive, the serum would contain 200 units of antitoxin in a cubic centimeter. These injections are best given with precision syringes, the one devised by Hitchens being particularly serviceable (Fig. 91). The syringes are sterilized and the needles are dipped in sterile vaselin to plug them. The mixtures are made in the barrel of the syringe and sufficient sterile salt solution is placed in the side arm to bring the total volume of the injection up to 4 c.c., and to wash in all traces of toxin and antitoxin. The mixtures are allowed to

stand for at least fifteen minutes (Park) before being injected (Fig. 92). The pigs must be of proper weight, *i. e.*, about 250 to 300 grams; the abdominal wall is shaved and the injection given directly in the median abdominal line. The animals are placed two in a cage, and carefully observed for four or five days for symptoms of toxemia and edema about the site of injection.

Römer and Sames' Method¹ of Determining Small Amounts of Diphtheria Antitoxin.

—The principle of this method is based upon the observation that, when very small amounts of diphtheria toxin are injected *intracutaneously* into the abdominal skin of guinea-pigs, small areas of edema and necrosis result in about forty-eight hours. When such injections are made with mixtures of toxin and antitoxin the presence of free toxin is indicated by such tissue changes. It is chiefly used in determining the antitoxin content of human serums after active immunization with the toxin-antitoxin mixtures of von Behring.

Technic.—I conduct this test in the following manner: The "limes-necrosis" (L_n) dose of a toxin is first determined, which is the amount of toxin which, together with $\frac{1}{1000}$ unit of standard antitoxin, will still produce a minimal amount of necrosis in forty-eight hours after *intracutaneous* injection into guinea-pigs. A series of dilutions of the L_n dose of

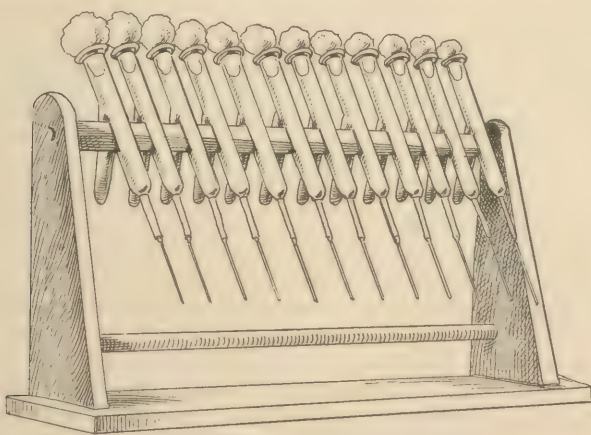


FIG. 92.—A BATTERY OF HITCHENS SYRINGES.

a toxin is made, ranging from 1 : 5 to 1 : 100, and 0.2 c.c. of each mixed with 0.2 c.c. of antitoxin so diluted that each 0.1 c.c. contains $\frac{1}{1000}$ of a unit. These mixtures are made in small test-tubes, the cotton stoppers paraffined, and the tubes incubated for three hours and placed in the refrigerator for twenty-one hours, after which 0.2 c.c. of each is injected into guinea-pigs (prepared by pulling out the hairs); several injections may be made in each pig.

When the L_n dose of the toxin has been determined this amount is mixed in a similar manner with varying amounts of the patient's serum being tested. The amount of serum just neutralizing the toxin contains $\frac{1}{1000}$ unit of antitoxin from which the amount of antitoxin per cubic centimeter of serum may be computed. For example, I have found that 0.003 c.c. of serum of a person reacting negatively to the Schick test neutralized this amount of toxin; therefore, each cubic centimeter of this person's serum contained 0.33 unit of antitoxin.

Kellogg's Method for Determining the Presence of Natural Antitoxin in Human Serum as a Substitute for the Schick Test.—Kellogg,² drawing attention to the practical difficulties of the Schick test for antitoxic immunity to diphtheria and especially the technical procedures surrounding the toxin, has advocated this guinea-pig intracutaneous test as a means for determining the presence or absence of antitoxin in human blood as a substitute for the Schick test. By means of his method it is claimed that specimens of blood may be sent to a laboratory and the work thereby centralized; Kellogg has given the following technic as employed in the California State Hygienic Laboratory; guinea-pigs are injected *intracutaneously* as in Römer's method:

¹ Ztschr. f. Immunitätsf., 1909, 3, 344.

² Jour. Amer. Med. Assoc., 1922, 78, 1782.

"One-three-hundredth minimal lethal dose of toxin will produce a definite sharp reaction, characterized by redness with some induration about 15 mm. in diameter, reaching its height in forty-eight hours and subsiding without necrosis. The reddened area fades quickly to a brownish color and there is some furfuraceous scaling of the skin.

"The smallest amount of toxin that will produce a definite necrosis of the skin is about $\frac{1}{40}$ minimal lethal dose. In the test a predetermined strength of toxin is mixed with an equal volume of blood-serum from the person to be tested, the mixture allowed to stand for half an hour at room temperature, and then 0.2 c.c. of the mixture is injected intracutaneously into the shaved skin of a white guinea-pig. The toxin used is standardized so that the amount contained in 1 c.c. is one-thirtieth of the L+ dose.

"Since the L+ dose of toxin is the amount that will neutralize one standard unit of antitoxin, leaving 1 minimal lethal dose of toxin free, it follows that 1 c.c. of one-thirtieth the L+ dose mixed with 1 c.c. of serum containing $\frac{1}{30}$ unit of antitoxin will leave a balance of $\frac{1}{30}$ minimal lethal dose, and 0.1 c.c. of each will have a surplus of $\frac{1}{300}$ minimal lethal dose of toxin. The volume of mixture injected, 0.2 c.c., therefore, represents one-three-hundredth of the L+ dose of toxin, plus whatever antitoxin may be in 0.1 c.c. of the serum.

"The reading is made in from forty-eight to seventy-two hours. If the serum tested contains exactly $\frac{1}{30}$ unit of antitoxin per cubic centimeter, the amount stated by Schick as being the minimal protective amount, $\frac{1}{300}$ minimal lethal dose of toxin, will be free in the mixture and produce the reaction as noted above.

"If the amount of antitoxin is greater, even by so small a margin as $\frac{1}{100}$ unit, no reaction will appear. If the amount of antitoxin is less than $\frac{1}{30}$ unit, even as much less as $\frac{1}{100}$, superficial necrosis will be plainly evident.

"The reason for the sharpness of these differences with such small amounts of antitoxin can be appreciated when it is recalled that the L+ dose of toxin (which just kills a 250-gram pig when mixed with 1 unit of antitoxin) may contain more than 100 minimal lethal doses.

"A variation of $\frac{1}{17}$ minimal lethal dose one way or the other from the $\frac{1}{300}$ required to produce a definite reaction produces either necrosis or absolute lack of reaction, as the case may be."

PRODUCTION OF TETANUS ANTITOXIN

The method used in the production of tetanus antitoxin is similar to that employed in producing diphtheria antitoxin, the horses being inoculated with increasing doses of a strong tetanus toxin.

Tetanus Toxin.—The toxin is secured by inoculating large flasks or tubes of neutral veal broth containing 1 per cent. of sodium chlorid and peptone with abundant tetanus culture, and growing these anaerobically at 37° C. for two weeks. Anderson and Leake¹ prepare 100 liters of broth with 50 kilograms of minced round steak and add 0.5 per cent. sodium chlorid and 1 per cent. peptone; after steaming for an hour the reaction is made neutral to phenolphthalein and the broth filtered through paper into liter Erlenmeyer flasks, followed by steaming without pressure for one and a half hours. The broth may be stored for a period of two weeks or less. Just before inoculating a 1 per cent. solution of C. P. glucose, (powdered) is added and the medium again heated for one and a half hours without pressure, cooled to 40° C., and immediately inoculated a few centimeters below the surface with 1 c.c. of a twenty-four-hour broth culture of tetanus bacilli which has been subcultured daily in 1 per cent. glucose broth for one to three weeks. No oil or other means is used to secure anaerobiasis; incubation is allowed to go on undisturbed for fifteen days at 37° C. The cultures are then filtered rapidly through Berkefeld filters, and the toxin preserved in fluid form with the addition of 0.5 per cent. phenol. As previously mentioned, the toxin rapidly deteriorates—especially tetanospasmin—and for purposes of antitoxin standardization it is usually preserved in a dry state after being precipitated with ammonium sulphate. The yellowish, crystalline masses are readily soluble in water or salt solution, and should be used immediately after solution takes place. The strength of the toxin is determined by injecting increasing amounts into white mice or 350-gram guinea-pigs.

¹ Jour. Med. Research, 1915, 33, 239.

Immunizing the Animals.—According to Park, the “horses receive 5 c.c. as the initial dose of a toxin, of which 1 c.c. kills 250,000 grams of guinea-pig, and along with this twice the amount of antitoxin required to neutralize it. In five days this dose is doubled, and then every five to seven days larger amounts are given. After the third injection the antitoxin is omitted. The dose is increased at first slowly until appreciable amounts of antitoxin are found to be present, and then as rapidly as the horses can stand it, until they support 700 to 800 c.c. or more at a time. This amount should not be injected in a single place, or severe local and perhaps fatal tetanus may develop, or immunization may be conducted in exactly the same manner as described for diphtheria except that one only increases each dose by 50 per cent. Horses withstand the effects of tetanus toxin better than diphtheria toxin. Good horses yield a serum containing 200 to 600 units per cubic centimeter” (Park).

Collecting the Serum.—The horses are bled, and the serum is collected under strict aseptic precautions, in a manner similar to the collection of antidiphtheric serum. The serum should be clear and free from blood, and should be proved sterile by cultural tests. It may be preserved in the liquid state by adding 0.5 per cent. of phenol or 0.4 per cent. of tricresol.

Standardizing the Serum.—The official immunity unit of tetanus antitoxin of the United States Government is based largely upon the work of Rosenau and Anderson. These investigators, together with a Committee of the Society of American Bacteriologists, have defined the unit of tetanus antitoxin to be “*ten times the least amount of serum necessary to save the life of a 350-gram guinea-pig for ninety-six hours against the official test dose of a standard toxin.*” This test dose consists of 100 minimal lethal doses of a precipitated and dried toxin, tested out against 350-gram pigs, and preserved in the Hygienic Laboratory from where it is sent to various antitoxin plants for the purpose of securing a uniform method and unit of standardization.

In standardizing tetanus antitoxin the L_+ dose of toxin is employed. A standard toxin and an antitoxin, arbitrary in their first establishment, are preserved in the Hygienic Laboratory, and are kept constant by making frequent tests one against the other. In determining the L_+ dose, increasing amounts of toxin are mixed with a constant amount of antitoxin equal to one-tenth of an immunity unit, and injected into 350-gram pigs. The L_+ dose must contain just enough toxin to neutralize this amount of antitoxin and kill a pig in four days. This L_+ dose of toxin is sent out by the Hygienic Laboratory to those interested, commercially or otherwise, in the manufacture of antitoxin for purposes of standardization.

For determining the strength of an unknown serum a large number of mixtures are made, each containing the L_+ doses of the toxin and increasing quantities of antitoxin. The measurements are made with accurate volumetric pipets, and the total volume brought up to 4 c.c. with sterile salt solution in order to equalize concentration and pressure. The mixtures are allowed to stand at room temperature for an hour, and are then injected subcutaneously into 350-gram pigs. This method of titrating the antitoxin is shown in the following example from Rosenau and Anderson:

TABLE 2.—METHOD OF TITRATING TETANUS ANTITOXIN

No. OF Pig.	WEIGHT OF PIG IN GRAMS	SUBCUTANEOUS INJECTION OF A MIXTURE OF—		TIME OF DEATH.
		Toxin (Test Dose).	Antitoxin.	
		Gram.	C.c.	
1.....	360	0.0006	0.001	Two days, four hours.
2.....	350	0.0006	0.0015	Four days, one hour.
3.....	350	0.0006	0.002	Symptoms.
4.....	360	0.0006	0.0025	Slight symptoms.
5.....	350	0.0006	0.003	No symptoms.

In this series the animal receiving 0.0015 c.c. of antitoxin died in approximately four days; this amount of serum, therefore, represents $\frac{1}{10}$ of one unit.

BOTULINUS ANTITOXIN

The nature of the botulinus poison has previously been described. Wassermann has recently immunized horses against this toxin, and the antitoxin shows unmistakable value in animal experiments, although it has not been employed frequently enough in this form of poisoning in human beings to prove its value.

ANTIDYSENTERIC SERUM

The Kruse-Shiga type of dysentery bacillus has been shown to produce varying amounts of a soluble toxin; and antiserums, which are partly antitoxic and partly bactericidal in nature, have been prepared, and have apparently yielded good therapeutic results in the hands of several observers. Potent antiserums for the Flexner type of bacillus and for various strains isolated from the feces of cases of infantile ileocolitis have not been produced. Even a virulent strain of the dysentery bacillus does not produce true soluble toxins in a manner comparable to those produced by tetanus and diphtheria. Potent toxins are seldom secured with less than two to three weeks' incubation, and fresh cultures of whole or autolyzed bacilli are likewise quite too toxic, indicating that although a soluble toxin may be produced, considerable endotoxin is also present in the bacilli.

Antidysenteric serum has very little prophylactic value, but in individual cases it frequently exerts a curative action, and should be available for use in institutions and armies when dysenteric infection is prevalent.

The older investigators, such as Kruse and Shiga, produced antiserums by immunization with whole bacilli. Later Kraus and Doerr prepared antitoxic serums with the toxin alone. At the present time the evidence would seem to indicate that the best serums are prepared by injecting both toxins and bacilli, producing a serum that is essentially antitoxic and bactericidal in action.

Culture.—Young and healthy horses are best adapted for immunization. Two methods may be followed: (1) Immunization with toxin, or (2) with young cultures of whole bacilli. As previously mentioned, investigations have tended to show that the most potent serums are secured by using mixtures of both toxin and micro-organisms.

Several strains of dysentery bacilli should be used in order that a poly-

valent serum may be prepared. Cultures should be grown for two weeks at 37° C., in alkaline broth similar to that used for preparing diphtheria toxin; this should be neutralized to phenolphthalein, and 7 c.c. normal soda solution to a liter added. The minimal lethal dose of the mixed *unfiltered* cultures is determined by giving young rabbits increasing doses intravenously in order to obtain a guide as to the proper dose for immunization. Fatal doses produce severe diarrhea and paralysis of the extremities, with rapid loss in weight. Rabbits and horses are quite susceptible to the toxin; guinea-pigs and mice are more resistant.

TABLE 3.—METHOD OF DETERMINING THE MINIMAL LETHAL DOSE OF DYSENTERY CULTURE

No.	WEIGHT, GRAMS	DOSE IN C.C.	RESULT.
1.....	710	0.025	No symptoms.
2.....	690	0.05	No symptoms.
3.....	695	0.1	Diarrhea. Recovered.
4.....	690	0.2	Death third day.
5.....	700	0.3	Death second to third day.

In this instance the minimal lethal dose was 0.2 c.c. and subsequent cultures of the same strains, grown under similar conditions, showed this dose to remain quite constant.

It is good practice to keep the cultures growing during the entire time of immunization. Cultures may, however, be grown for three weeks, filtered through porcelain, and with the addition of 0.5 per cent. phenol, the toxin preserved for long periods of time. The minimal lethal dose of such a toxin is determined in the manner directed above.

Immunizing the Animals.—Since horses are quite susceptible, the initial dose of unfiltered and unheated culture should not be larger than the minimal lethal dose for a young rabbit. The dosage is gradually increased, and the injections are given subcutaneously for from four to six months, after which several injections of from 300 to 350 c.c. may be given intravenously at one time. If at any time diarrhea and other symptoms of toxemia are well marked, subsequent doses should be smaller and should be given at longer intervals until a higher immunity is produced.

Instead of using bouillon cultures, young agar cultures may be used, the bacilli being grown for seventy-two hours, and one-tenth of an ordinary slant being given as the first dose. The early doses are heated to 60° C. for an hour and injected subcutaneously; the later doses consist of cultures washed from 30 to 40 tubes, and are given intravenously.

Flexner and Amoss's Method for Rapid Production of Antidysenteric Serum.¹—By this method potent antidysenteric sera can be safely prepared in the horse by the method of three successive intravenous injections of living cultures or toxin with intervening rest periods of seven days; and effective serum for therapeutic purposes may be prepared in about ten weeks. By inoculating alternately living bacilli belonging to the Shiga and Flexner groups a polyvalent serum of high titer may be secured which should be suitable for the serum treatment of acute bacillary dysentery, irrespective of the particular strain or strains of the dysentery bacillus causing the infection.

Cultures are grown upon agar-agar slant surfaces in tubes 15 x 160 mm. in size for twenty-four hours, and the growth in each tube suspended in

¹ Jour. Exper. Med., 1915, 21, 515.

2 c.c. of salt solution. Horses are injected intravenously; the first dose is 1 c.c. of the suspension of Flexner bacilli after heating to 60° C. for thirty minutes; on each of the following two days 5 c.c. of heated suspension are usually given, followed by a rest of seven days, when living cultures are injected. The temperature is taken daily and used as an index of the reaction and subsequent doses. With the living bacilli the doses injected on each of three days in succession are 4, 10, and 30 loopfuls suspended in salt solution. Flexner and Shiga bacilli are inoculated alternately on three successive days, with intervening rest intervals of seven days, the doses being chosen so as to produce a sharp febrile reaction which subsides in twenty-four hours. At the end of eight to ten weeks' immunization the serum contains immune agglutinins and a well-marked degree of antibacterial and antitoxic value.

Collecting and Testing the Serum.—After three or four months a trial bleeding should be made and the serum tested as follows: The minimal lethal dose of a culture is determined and ten times this amount placed in a series of tubes or syringes with increasing doses of serum; the total quantity of injection is made up to 4 c.c. with sterile salt solution. The mixtures are set aside for one hour at 35° C. and injected intravenously in young rabbits. The animals are to be observed for at least five days for diarrhea, paralysis, and loss in weight. For determining the antitoxic value a toxin is prepared by cultivating the bacilli in sugar-free broth containing calcium carbonate for three days; the bacilli are then killed with ether; the ether is removed and the culture filtered through hard paper or a Berkefeld filter and the toxin kept in the refrigerator.

TABLE 4.—METHOD OF TESTING ANTIDYSENTERIC SERUM (KRUSE-SHIGA)

No.	WEIGHT, GRAMS	CULTURE, 0.2 C.C. M. L. D. C.C.	SERUM C.C.	RESULT.
1.....	600	2.0	0.00025	Died second day.
2.....	610	2.0	0.0005	Died third day.
3.....	615	2.0	0.001	Diarrhea, recovered.
4.....	590	2.0	0.002	Diarrhea, paralysis.
5.....	600	2.0	0.004	No symptoms.
6.....	590	2.0	0.006	No symptoms.

In this instance 0.004 c.c. of serum was sufficient to protect young rabbits against ten fatal doses of culture, and demonstrated that it is possible to secure a fairly potent serum against the toxins of the Kruse-Shiga micro-organism.

According to Todd, if the antiserum is given at least one-half hour after administering the culture, it will protect the rabbit. If given twenty-four hours later, it affords no protection. Similarly, the mixtures of culture and serum must not be injected immediately after mixing, as the results are more irregular than if they are allowed to stand for one-half to one hour before injecting.

If the trial bleeding shows a satisfactory serum the horse is bled aseptically, as was previously described, and the serum is separated and preserved with 0.5 per cent. phenol in quantities of 10 c.c. in sterile containers. As there is no official immunity unit, the serum is administered in doses of from 5 to 10 c.c. until a therapeutic effect is secured.

ANTISTAPHYLOCOCCUS SERUM

Both *Staphylococcus pyogenes aureus* and *S. pyogenes albus* have been shown to produce certain soluble toxins, such as a leukocidin and a hemolysin, which are partly responsible for the tissue destruction and symptoms that accompany these infections. Severe staphylococcus infection is probably due in part to the paralyzing effect and actual destructive action of the leukocidin upon the leukocytes, preventing for the time being the walling off of the lesion and effectual phagocytosis. Antistaphylococcus serums have been shown to counteract the action of the leukocidin and the hemolysin, and may be useful in the treatment of severe, spreading, or metastatic staphylococcus infections.

According to Neisser and Wechsberg, during staphylococcus disease an antihemotoxin is produced against the hemotoxin of the cocci; later Bruck, Michaëlis, and Schulze attempted to show that a demonstration of this antistaphylolysin in the serum may be regarded as evidence of a staphylococcus infection.

Preparation of Antistaphylococcus Serum.—For immunization purposes several different cultures of the *Staphylococcus aureus* should be used in order that the antisera may be polyvalent. Goats or horses may be employed. Cultures may be grown on neutral agar for forty-eight hours, and an emulsion, equivalent to half an agar slant, heated to 60° C. for one hour and injected subcutaneously in an adult goat. If 10 different strains are used a 4-mm. loopful from each culture, emulsified in 5 c.c. of sterile salt solution, will be about the proper dose for the first injection. Subsequent doses are given at intervals of a week, and are rapidly increased in size until full, living, unheated cultures are injected intravenously without harm to the animal. The serum may be tested by determining its content of antilysin or of bacteriotropin. Complement-fixation tests are occasionally useful for obtaining an insight into the quantity of bacteriolysin present.

Technic of the Antilysin Test.—The object of this test is to determine the amount of antihemolysin present in a serum, which is dependent on the amount of serum necessary to protect the red blood-cells of rabbits against a solution of the staphylolysin.

(a) *Staphylolysin.*—This is prepared by growing a known hemolysin-producing staphylococcus in slightly alkaline broth for three weeks, filtering through a Berkefeld filter, and preserving the filtrate with 0.5 per cent. phenol in the refrigerator.

(b) *Rabbit Blood.*—Remove 2 or 3 c.c. of blood from the ear of a rabbit and place in 5 c.c. of a 1 per cent. sodium citrate in normal salt solution. Wash the corpuscles three times, and make up in a 1 per cent. suspension (dose 1 c.c.) or up to the original volume of blood (dose, 1 drop).

(c) *Patient's Serum.*—The serum is inactivated by heating to 56° C. for half an hour.

(d) *Control Serum.*—As every normal serum contains a certain amount of antilysin, it is necessary to use a normal control serum. Normal horse-serum, dried *in vacuo* to prevent deterioration, and freshly dissolved for each test in 10 volumes of sterile distilled water or salt solution, has been advocated by Bruck, Michaëlis, and Schulze.

(e) *The Test.*—It is first necessary to titrate the staphylococcus filtrate to ascertain the amount of lysin present. This is accomplished according to the following scheme:

TABLE 5.—METHOD OF TITRATING STAPHYLOLYSIN

AMOUNT OF STAPHYLOLYSIN FILTRATE.	RABBIT BLOOD 1 PER CENT.	NORMAL SALT SOLUTION.	RESULT OF HEMOLYSIS AFTER TWO HOURS AT 37° C. AND TWENTY-FOUR HOURS IN REFRIGERATOR.
0.005 c.c.....	1 c.c.	q. s. 2 c.c.	No hemolysis.
0.001 c.c.....	1 c.c.	q. s. 2 c.c.	No hemolysis.
0.02 c.c.....	1 c.c.	q. s. 2 c.c.	Slight hemolysis.
0.05 c.c.....	1 c.c.	q. s. 2 c.c.	Marked hemolysis.
0.1 c.c.....	1 c.c.	q. s. 2 c.c.	Complete hemolysis.
0.2 c.c.....	1 c.c.	q. s. 2 c.c.	Complete hemolysis.
0.5 c.c.....	1 c.c.	q. s. 2 c.c.	Complete hemolysis.
1.0 c.c.....	1 c.c.	Complete hemolysis.

In this test 0.1 c.c. is the smallest amount of lysin that can completely hemolyze the given quantity of erythrocytes, and is taken as the unit for the second part of the test.

The lytic dose of filtrate just determined is now placed in a series of small test-tubes, with increasing doses of serum to be tested and a constant dose of corpuscles.

TABLE 6.—METHOD OF TITRATING ANTISTAPHYLOLYSIN IN A SERUM

AMOUNT OF FILTRATE.	INACTIVATED SERUM.	1 PER CENT. RABBIT CORPUSCLES.	NORMAL SALT SOLUTION.	READINGS AFTER INCUBATION AT 37° C. FOR TWO HOURS AND TWENTY-FOUR HOURS IN THE REFRIGERATOR.
0.1 c.c.....	0.001 c.c.	1 c.c.	q. s. 2 c.c.	Complete hemolysis.
0.1 c.c.....	0.005 c.c.	1 c.c.	q. s. 2 c.c.	Slight inhibition of hemolysis.
0.1 c.c.....	0.01 c.c.	1 c.c.	q. s. 2 c.c.	Marked inhibition of hemolysis.
0.1 c.c.....	0.05 c.c.	1 c.c.	q. s. 2 c.c.	Complete inhibition of hemolysis.
0.1 c.c.....	0.1 c.c.	1 c.c.	q. s. 2 c.c.	Complete inhibition of hemolysis.
0.1 c.c.....	0.2 c.c.	1 c.c.	q. s. 2 c.c.	Complete inhibition of hemolysis.

In this instance 0.05 c.c. of the patient's serum was sufficient completely to neutralize the lysin.

A similar test is carried out with normal horse-serum. The antilytic dose of this serum is taken as 1, and the patient's serum is compared with this unit. For example, if 0.1 c.c. of normal horse-serum was sufficient to neutralize the lysin in this experiment, then the antilysin value of the patient's serum is 2.

According to Arndt and others, a high antilysin content of a serum is to be regarded as indicating a staphylococcic infection, even if it is impossible to establish fixed limits for the values.

PRODUCTION OF ANTIVENIN

Snake venom contains two toxins, one being largely neurotoxic and producing paralysis of the respiratory centers, and the other being hemotoxic and irritant, and producing local necrosis of tissues, hemolysis, etc. In venom poisoning the neurotoxic effect is most dangerous. Largely as the result of the work of Calmette and Fraser an antivenin has been prepared that is capable of counteracting the neurotoxic action not only of cobra venom, but to a lesser extent of other venoms as well. These serums, however, appear to have no effect or but very little upon the irritant toxins.

In the poisonous American snakes, such as the rattler, moccasin, and copper-head, the effects of the irritant toxins are largely in evidence, and satisfactory antiserums for these venoms have not been prepared (McFarland).

In preparing antivenins the toxins, since they are thermolabile, must be used unheated; subcutaneous injections are usually followed by extensive sloughing, and although a certain amount of immunity may be induced in the horse by intravenous injection, there is apparently no protection against the local action of the toxins.

Preparation of Antivenin.—According to Calmette, horses may be immunized by giving them weekly subcutaneous injections of gradually increasing doses of cobra venom, heated to 70° C., for an hour which precipitates the irritant toxins without injuring the neurotoxin. The initial dose is usually 0.01 gram, gradually increased, until by the end of four months 4 grams may be given at a single dose. The serum is then tested by mixing increasing doses with the minimal lethal dose for a young rabbit, and injecting the mixtures intravenously into a series of rabbits.

Since the neurotoxin may prove dangerous in any case of snake bite, antivenin may be given to advantage, although the local pain and necrosis are not relieved by the serum.

Flexner and Noguchi have successfully immunized rabbits and dogs with rattlesnake venom which had been treated with hydrochloric acid and iodine trichlorid, which deprived the venom of a large part of its toxicity while still preserving the power of causing the production of antivenin. Anticrotalus venom was found without appreciable antitoxic power for cobra and daboia venoms, and but feeble antitoxic activity for the water-moccasin venom.

PRODUCTION OF POLLEN ANTITOXIN

The pollen of certain plants is markedly toxic for susceptible individuals. In America the pollen of the golden-rod and of rag weed frequently produce a syndrome of distressing symptoms known as "autumnal catarrh." The onset and character of the symptoms of pollen intoxication are strongly suggestive of an anaphylactic reaction. Dunbar has studied pollen toxins quite extensively, and considers them the etiologic factor in the production of hay-fever.

Pollen antitoxin has been prepared by immunizing susceptible horses, the toxin being isolated by mixing the ground pollen with 5 per cent. sodium chlorid solution and 0.5 per cent. phenol at 37° C. for ten hours. In the form of a protein, it is then precipitated by adding eight to ten volumes of 96 per cent. alcohol, dissolving the resultant white precipitate in physiologic salt solution (Citron).

THE MEASURE OF ANTITOXINS

Antitoxin Unit.—*A unit is the definite measure of antitoxin in any serum or solution that will neutralize a certain amount of toxin.* Originally the unit of diphtheria antitoxin was determined according to the method of Behring and was defined as ten times the least quantity of serum that protected a 300-gram guinea-pig against ten times the least certainly fatal dose of toxic bouillon. This method proved unsatisfactory on account of variations in the toxin as pointed out by Ehrlich, who showed that this toxin does not possess uniform combining affinity for the antitoxin. Ehrlich, therefore, devised a method by which a standard dried toxin was produced which is now widely used and described in the preceding pages. As previously stated the United States Government has established a definite unit for

the standardization of diphtheria and tetanus antitoxins, and frequently examines the serums made by various licensed manufacturers. Officers of the Public Health and Marine Hospital Service purchase from reliable pharmacists several grades of antitoxins made by each manufacturer, which are then sent to the Hygienic Laboratory at Washington, where they are tested for potency, freedom from contamination by bacteria, chemical poisons, especially tetanus toxin, and for excessive amounts of preservative. Delinquencies are reported immediately, and steps are taken to withdraw that particular lot of serum from the market.

A unit of diphtheria antitoxin may be defined as the "amount of antitoxin that will just neutralize 100 minimal fatal doses of toxin for a 250-gram guinea-pig."

A unit of tetanus antitoxin may be defined as the "amount of antitoxin which will just neutralize 1000 minimal fatal doses of toxin for a 350-gram guinea-pig."

The standardization of these serums is useful as a guide to their administration, especially when given for prophylactic purposes, where experience has taught that so many units usually confer protection; it also serves for purposes of record. In the treatment of diphtheria and tetanus, however, the serums are usually given until a therapeutic effect is noted, regardless of the number of units administered. If it were possible to determine quickly and accurately the amount of toxin in a given patient, then neutralization could be accomplished along the same lines that make this possible in the test-tube. The indications are to administer at once sufficient antitoxin to neutralize all the toxin, giving subsequent doses large enough to overcome the toxin as it is produced until the focus of infection is removed.

Antitoxin should be kept in a cold place and protected from air and light. When this is done, they usually do not deteriorate more than 30 per cent. of their original strength, and often much less, within a year. All manufacturers place a large number of units in the container than the label calls for, in this way allowing for the gradual loss in strength up to the date specified on the label. According to Park the antitoxin in old serum is just as effective as that in fresh serum, except that there is less of it.

PRACTICAL APPLICATION

The employment of antitoxic serums, both in prophylaxis and in the treatment of infection, is considered in greater detail in the chapters on Passive Immunization and Serum Therapy.

CHAPTER XIV

FERMENTS AND ANTIFERMENTS

Ferments.—The term “ferment” introduced in relation to infection and immunity has proved very confusing. Owing largely to the investigations of Vaughan, Abderhalden, Jobling, and their associates this term has come into very general use, but in some instances appears to have been ill chosen.

It is well known that some bacteria contain or elaborate true ferments or enzymes; also that an increase of true ferments like the proteases may be found in the plasma in pathologic conditions. Jobling has used the term in this strict meaning and in conformity with our general knowledge of true enzymes or ferments.

Vaughan, Abderhalden, and others, however, have applied the term to other substances in serum which have not been clearly differentiated from the lytic antibodies or amboceptors and complement. Of course, complement may be a ferment, but this has not been definitely proved. And the use of the word “ferment” as practically synonymous with antibody, has resulted in confusion with the true enzymes or ferments, as proteases, lipases, and others.

Bacterial Ferments in Relation to Infection.—While many pathogenic bacteria are known to produce true ferments or enzymes, those of *Bacillus pyocyaneus* and the pneumococcus have received most attention in relation to the production of disease.

Bacillus pyocyaneus contains and produces a particularly active proteolytic ferment which has been studied by Emmerich and Löw,¹ and to which they have given the name *pyocyanase*. These investigators have claimed that this ferment is capable of agglutinating and digesting not only *B. pyocyaneus* but other bacteria as well, including anthrax, typhoid, diphtheria, and cholera bacilli. On the basis of the positive results of *in vitro* agglutination and bacteriolytic tests with pyocyanase, they advocated the use of this enzyme for the treatment of bacterial infections by local application and subcutaneous injection. According to these experiments proteolytic bacterial ferments may reduce infection by direct destruction of the infecting bacterium.

Fermi,² Petrie,³ and Dietrich,⁴ however, were unable to substantiate these claims for the bacteriolytic activity of pyocyanase. In their opinion bacterial enzymes are unable to digest bacteria from which they are derived or bacteria of other kinds, and that, generally speaking, proteolytic ferments exert no action on living plant or animal cells. These investigators were inclined to believe that the morphologic and bactericidal changes produced by seeding bacteria in solutions of pyocyanase were brought about by osmotic changes or plasmolysis.

Proteolytic enzymes of pneumococci have been studied by Dick⁵ who found them in the blood during pneumonia about the time of crisis, and

¹ Ztschr. f. Hyg., 1899, 31, 1; *ibid.*, 1901, 3691. Centralbl. f. Bakteri., 1900, 27, 1.

² Ztschr. f. Hyg., 1894, 18, 83.

³ Jour. Path. and Bacteriol., 1903, 8, 200.

⁴ Centralbl. f. Bakteri., 1901, 30, 574.

⁵ Jour. Infect. Dis., 1912, 10, 383.

Rosenow,¹ who demonstrated that extracts of virulent pneumococci and filtrates of broth cultures contain proteolytic enzyme capable of hydrolyzing the proteins in broth and serum with a proportional increase of toxicity. Avery and Cullen² found an intracellular enzyme or enzymes in pneumococci capable of hydrolyzing to some extent intact protein and especially peptones; also endolipolytic and various carbohydrate-splitting ferments.

It is highly probable that the enzymes produced by pathogenic bacteria are for the primary purpose of nutrition and the production of favorable environmental conditions for multiplication. As shown by Diehl³ their production is greatly influenced by the conditions under which the bacteria are growing. In so far as their relation to infection is concerned bacterial ferments probably possess an important status for at least two reasons:

1. Ferments elaborated by bacteria aid in their nutrition and enable them to survive in the tissues, thereby aiding in the production of infection.
2. These ferments are probably able to bring about digestion of devitalized body and bacterial cells with the production of toxic substances capable of retarding phagocytosis, and when absorbed adding an element to toxemia.

On the other hand, these ferments and especially the products of digestion, are inimical to bacteria of the same and different species, thereby tending to sterilize local collections of pus and offering an explanation for the instances in which pus from long-standing lesions are found to be free of living bacteria.

The similarity of toxins to ferments has been previously discussed in the chapters on Infection.

Immunity to Bacterial Ferments; Antiblastic Immunity.—Since the various ferments produced by some pathogenic bacteria may play an important rôle in the production of infection and disease, the question naturally arises as to whether or not the body cells possess a means of defense.

Dochez and Avery⁴ have found that antipneumococcus sera exert an inhibitory influence upon the growth of pneumococci which they believe is directed against the ferments produced by these bacteria. "Pneumococcus in order to grow must obtain a sufficient supply of protein and carbohydrate; these substances are furnished by the environmental medium, but probably require to render them suitable for absorption preliminary preparation in the nature of digestion. This change is effected at the surface of the bacterial cell and the integrity of this digestive zone is essential to the growth of the bacterium. Anti-enzymotic bodies such as have been demonstrated in immune serum act at the point of contact of the cell with its environment, and influence in an unfavorable manner the nutritional processes there carried on, and the consequence of such action is retardation or inhibition of growth." To this possible type of immunity Dochez and Avery have applied the term "antiblastic immunity" indicating antagonism to the growth activities of a micro-organism. The term was coined by Ascoli⁵ several years ago to explain the action of antianthrax serum inhibiting the metabolic activities of anthrax bacilli, and particularly the formation of capsules.

Similar investigations against the ferments of other bacteria do not appear to have been made except by Gheorghiewsky,⁶ who found immune

¹ Jour. Infect. Dis., 1912, 11, 286.

² Jour. Exper. Med., 1920, 32, 547, 571, 583.

³ Jour. Infect. Dis., 1919, 24, 347.

⁴ Jour. Exper. Med., 1916, 23, 61.

⁵ Centralbl. f. Bakteriöl., orig., 1908, xlvii, 178.

⁶ Ann. de l'Inst. Pasteur, 1899, 13, 298.

serum inhibited pigment production by *Bacillus pyocyaneus*, and by von Dungern,¹ who found that immune serum may inhibit the liquefaction of gelatin by *Staphylococcus aureus*. Further researches may show that ferments greatly aid bacteria in infection and that forces may be mobilized by the animal body in opposition to them.

Leukocytic and Serum Ferments in Relation to Infection.—In addition to the true ferments elaborated by bacteria either as exogenous ferments or as endoferments released upon disintegration of the bacterial cell, the serum during infection and disease is found to contain additional ferments probably derived from the body cells.

Vaughan regards these ferments as called out by the bacterial protein and more or less specific for this protein; in his theory of infection and immunity the ferments are believed to split the bacterial protein with the formation of a toxic and a non-toxic portion.

Jobling and Peterson² have studied the leukocytic and serum ferments in relation to infection with particular care and in an exhaustive manner. They have never confused these ferments with antibodies and have adhered strictly to chemical methods in their studies. They regard digestion of foreign proteins as the basis of resistance to and recovery from bacterial invasion and infection; for overcoming intoxication not due to the soluble or true exotoxins, they look to the cells or fluids of the body for the elaboration of true ferments capable of digesting toxic protein fragments to their lowest degradation and non-toxic products. The importance of these serum ferments to infection is, therefore, in direct relation to the part played by the bacterial proteins and protein-split products in pathologic processes.

Of these ferments the proteolytic ferments are of most importance in breaking down toxic complexes to non-toxic forms. Petersen³ has described several in serum as follows: (1) Leukoproteases embracing (a) an alkaline active ferment capable of splitting native protein largely to the proteose stage; (b) an acid-active ferment with a similar range of activity; (c) and ereptase, active in both acid and alkaline reaction and splitting partially hydrolyzed proteins to the amino-acid stage. These ferments are derived from disintegrating, but not living leukocytes. (2) Serum protease: a polyvalent, trypsin-like ferment, active in neutral or slightly acid or alkaline reactions. When antiferment is removed it is able to digest any native protein to the amino-acid stage. (3) Serum peptidase: a polyvalent ferment active under the same conditions as serum protease even in the presence of antiferment and capable of hydrolyzing proteins to the amino-acid stage.

Of these three ferments peptidase is regarded as most important inasmuch as it is not influenced by changes in antiferment, and is of the nature of a detoxicating agent hydrolyzing toxic albumoses and peptones to the non-toxic amino-acids.

Immunity to Leukocytic and Serum Ferments; Nature of Antiferments.—

According to some investigators, antiferments are to be found in large amounts in all normal serums, and are probably vitally concerned in the processes of life in preventing autodigestion. That they may be increased in number artificially by immunization up to a certain limit has been disputed; it is certain that they never attain the extreme amounts possible with the injection of toxins. This may be due to the formation of anti-antienzymes, produced by a regulating mechanism that prevents antienzymes from accumulating beyond a certain point and interfering with

¹ *Centralbl. f. Bakteriol., orig.*, 1898, 24, 710.

² *Jour. Exper. Med.*, 1912-16, 16-23.

³ *Archiv. Int. Med.*, 1917, 20, 515.

nutrition. It is possible that the body mechanism exerts a strict regulating effect between the formation of enzymes and antienzymes. Furthermore, when free receptors, such as normal antienzymes, are present in the body fluids, the body cells are not stimulated to produce these antienzymes in excess, nor does the presence of the free receptors stimulate the cells to produce antibodies against their normal side chains.

Many investigators claim to have produced antiferments experimentally. Morgenroth¹ believed that he obtained a specific antirennin by inoculating goats with rennin. Sachs² and Achaline³ assert that they have produced specific antipepsin or antitrypsin by inoculating animals with these ferments. Antisteapsin and antilactase have been prepared by Schutze,⁴ antityrosinase by Gessard,⁵ and antiurease by Moll.⁶

Jochmann and Müller have demonstrated the presence of an antiferment in the serum used against leukocytic ferments in diseases associated with great destruction of the leukocytes. Following these observers, Marcus Brieger and Trebing⁷ found that 90 per cent. of the patients suffering from carcinoma or sarcoma examined by them showed an increase of antitrypsin in the blood. Von Bergmann and Meyer⁸ confirmed this observation, although they found that a similar increase also occurred in 24 per cent. of non-cancerous patients. More recent work would indicate that the antitrypsin may be present in acute infections, such as pneumonia, typhoid fever, etc., in chronic infections, such as tuberculosis and syphilis, in exophthalmic goiter, and in severe anemias. As previously mentioned, Schwartz,⁹ Sugimoto,¹⁰ and Jobling and Peterson¹¹ believe that the antitryptic influence of blood-serum is due to the lipoids, and especially to the compounds of the unsaturated fatty acids.

The tryptic ferment liberated by disintegrating leukocytes and connective-tissue cells is largely responsible for the liquefaction of these cells and the formation of pus, as in abscess formation and autodigestion of infected surface wounds. On the other hand, an antitrypsin-like substance tends to limit the activities of the ferment and protect the surrounding tissues from progressive destruction. A deficiency of this substance may account for the rapid breaking down of infected glands and of a walled-off tuberculous lesion, the development of carbuncles, etc. A study of the antitryptic power of the blood may, therefore, prove of value in suppurative processes and in malignant disease, and considerably influence a prognosis.

The nature of antiferment or antienzyme has been the subject of investigation for many years, and with the result that many different theories have been advanced. For example, different protein fractions of the serum have been regarded as antitryptic by Landsteiner, Oppenheimer and Aaron, Cathcart, and more recently Fujimoto¹²; Baylis and Starling, Abderhalden and Gigon, and Rosenthal regarded the products of tryptic activity and especially the amino-acids as inhibiting ferment activity. Delezenne and Pozarski¹³ found that serum preserved with chloroform soon lost its anti-

¹ Centralbl. f. Bakteriöl., 1899, xxvi, 349.

² Fortschr. d. Med., 1902, 20, 425.

³ Ann. de l'Inst. Pasteur, 1901, xv, 737.

⁴ Zeitschr. f. Hygiene, 1904, 48, 457; Deutsch. med. Wochen., 1904, 30, 308.

⁵ Ann. de l'Inst. Pasteur, 1901, 15, 593.

⁶ Hofmeister's Beitr., 1902, 2.

⁷ Berl. klin. Wchnschr., 1908, xlv, 1349.

⁸ Berl. klin. Wchnschr., 1908, xlv, 1673.

⁹ Wien. klin. Wchnschr., 1909, xxii, 1151.

¹⁰ Arch. f. Exper. Path. u. Pharmacol., 1913, lxxii, 374.

¹¹ Jour. exper. Med., 1914, xix, 239, 459.

¹² Jour. Immunology, 1918, 3, 51.

¹³ Compt. rend. Soc. de biol., 1903, 55.

tryptic activity; Schwartz¹ found that the antitryptic action of normal serum is due to the lipoids of the serum which are partially removed by extraction with ether. Similar results were reported by Sugimoto² and this brings the subject down to the excellent work of Jobling and Petersen,³ who have identified the antiferments of serum with the unsaturated fatty acids.

According to these investigations an antiferment is not an antibody in the immunologic sense, but consists of the highly dispersed unsaturated lipoids of the serum and lymph. The amount of antiferment in a serum would depend, therefore, upon the amount of lipoids present, the degree of dispersion, and the degree of unsaturation. Lessening the dispersion by acidifying, salting, or heating inactivates the antiferment; physical absorption of the lipoids removes antiferment as likewise solution in chloroform and other lipid solvents. They believe that the antiferment lipoids are in more or less intimate physical combination with the serum albumin with which fraction they are thrown down by the usual methods of separation of the serum proteins; in all probability this accounts for the earlier views that antiferment resided in certain proteins of serum.

According to Jobling and Petersen antiferment is greatly increased during the acute infectious diseases, in pregnancy, in carcinoma, and cachexia; also in degenerative lesions of the central nervous system, after intravenous injection of proteins and in various other conditions. The antiferment of the lymph-stream is appreciably increased after feeding, while in starvation there is a progressive decrease.

Removal of the antiferment (unsaturated fatty acids of the serum) by any of the physical or chemical means mentioned above results in increased activity of the proteolytic ferments which may attack and digest various proteins including those of the serum, with the production of toxic split proteins.

Ferments vs. Antibodies in Disease; So-called "Protective Ferments" in Pregnancy and Disease.—It is largely to the researches of Abderhalden and his associates that we owe our knowledge of the fact that when food-stuffs are introduced into the body parenterally, *i. e.*, by subcutaneous or intravenous injection, ferments are produced that, by process of cleavage and reduction, deprive them of their individuality.

For example, as shown by Weinland,⁴ normal dog serum cannot reduce cane-sugar, whereas the serum of a dog immunized by several injections of this sugar is able to reduce it *in vitro* by means of a specific ferment of the nature of invertin. Similarly, normal serum is unable to cleave edestin (vegetable albumin), whereas the serum of an immunized dog will split this protein into simpler substances.

After he had proved experimentally that the animal organism is able to mobilize ferments against foreign substances Abderhalden next took up the question whether ferments are produced when substances native to the body but foreign to the blood are introduced into the circulation. Having learned from the researches of Veit, Schmorl, Weichard, and others that during pregnancy syncytial cells frequently enter the maternal circulation, Abderhalden used the serums of pregnant animals, and found that they contained a ferment-like substance capable of splitting placental peptone into amino-acids and coagulated placenta into peptones, polypeptids, and amino-acids.

¹ Wien. klin. Wchnschr., 1909, 22.

² Arch. f. exper. Path. u. Pharmacol., 1913, lxxiv, 14.

³ Jour. Exper. Med., 1914, 19, 459.

⁴ Ztschr. f. Biol., 1907, 279.

It was apparently thus established that the body cells are harmonically attuned to one another, and if new or modified cells or their products are brought into relation with other cells, they are received as foreign invaders, and their entrance is followed by the production of what Abderhalden has called "protective ferments" ("Abwehrfermente") capable of bringing about their cleavage into simpler products. In this manner the presence in the circulation of some of the body cells may give rise to the production of these ferments if the cells in question are really foreign to the blood-plasma and other cells.

Abderhalden has also stated that although he was led to make these investigations on the supposition that syncytial elements were present in the blood of pregnant women, it is not necessary that they be constantly in the blood, for every case of pregnancy has a complicated protein metabolism and there is a general exchange of substances between the placenta and the maternal blood that permits the entrance into the latter of protein products that have not been broken down completely into amino-acids, and that cause the organism to produce defensive proteolytic ferments.

In cancer, where the production of new cells is so marked, some of these cells or their products may easily be swept into the general circulation, where they act as foreign invaders and cause the formation of protective proteolytic ferments. It is a noteworthy fact, moreover, that the serum of carcinoma cases reacts best with carcinoma cells and that of sarcoma with sarcoma cells.

Similar ferments have been described in other conditions. Fauser has demonstrated that the blood-serum of dementia præcox patients contains ferments that act on the reproduction glands, so that the serum of males reacts with testicular extracts and that of females with ovarian extracts. These serums were, however, also found to react with thyroid tissue and brain cortex. In general paresis reactions were obtained with brain cortex and liver, also at times with thyroid gland, reproductive glands, and more rarely with kidney.

Abderhalden has found ferments for the tubercle bacilli in the blood-serum of tuberculous persons, and they have also been found in the blood-serum of syphilitics for the *Treponema pallidum*, either in pure culture or in organs containing large numbers of the parasites; Smith¹ has found a remarkable specificity of the ferments produced in rabbits following immunization with typhoid and paratyphoid bacilli and cocci.

The work of Abderhalden has been severely criticized and the existence of his "protective ferments" in pregnancy and disease denied. An enormous literature has accumulated on the application of his dialysis method in the diagnosis of pregnancy which has unfortunately detracted from interest and investigation in the fundamental principles.

Some investigators have denied the existence and specificity of these "ferments." However, that there may be an increase of true proteolytic ferments in pathologic conditions has been clearly established by Jobling and his associates as discussed above. The question is whether or not the "ferments" of Abderhalden are true ferments or a kind of antibody whose nature is unknown or which may be classed with the sensitizers acting with complement.

The researches of Van Slyke and his associates² employing Van Slyke's method of amino-nitrogen determination, have shown that practically every serum, whether from a pregnant or non-pregnant female, or from a

¹ Jour. Infect. Dis., 1916, 18, 14.

² Jour. Biol. Chem., 1915, 23, 377.

male, gave protein digestion when incubated with placenta tissue prepared according to the method of Abderhalden; further evidence of non-specificity was seen in the fact that carcinoma tissue was digested apparently to about the same extent as was placenta. Taylor and Hulton,¹ working with the protanin of salmon, found that normal serum and the serum of immunized animals gave about equal degrees of digestion, leading them to conclude that there is at present no reason for believing that the normal hydrolysis of the protein of the body occurs in the circulating blood, but that these metabolic changes presumably belong to the tissue cells.

The investigations of Sloan,² however, have shown that the serum in pregnancy may yield positive Abderhalden reactions without showing any evidences of digestion by Van Slyke's method. In the opinion of Sloan, the latter method is not applicable for determinations of ferment activity in which a non-soluble, moist, complex protein substance is incubated with serum in a test-tube, but without dialysis. My own experiments with Asnis and Frees agree with these findings. Sloan also found that the serum of animals injected with a suspension of placental cells did not give rise to an increase of ferments, but that the injection of split products of placental cells was followed by an increase of proteolytic ferments.

The work of Bronfenbrenner and his associates³ has also shown quite clearly that changes occur in the serum during pregnancy, with the production of a "ferment" or an antibody possessing a high degree of specificity. Too much excellent and careful work has been done to discard the fact that these substances may be found in the serum in pregnancy and pathologic conditions, although it is now equally well established that the nature of these substances and the mechanism of their activity are probably entirely different from the conceptions of Abderhalden.

Since these so-called "ferments" may not show their presence in chemical tests which are satisfactory for detecting the presence of such true ferments from leukocytes and serum as the proteases, the question of their nature, that is, whether they are true ferments or antibodies of the nature of amboceptors or cytolytins requiring the presence and activity of complement, becomes one of much interest and importance.

Unfortunately, this question cannot be definitely answered. Pearce and Williams⁴ were of the opinion that the "ferments" were not cytolytins, and Abderhalden has strenuously denied that his "ferments" and cytolytins were identical without, however, bringing forward conclusive proof.

The experiments of Stephan,⁵ Hauptmann,⁶ Bettencourt and Menezes,⁷ and Bronfenbrenner, however, have shown that complement bears an important relation to the activity of the so-called "protective ferments," which indicates that the latter may be of the nature of amboceptors. It would appear that in the light of our present knowledge at least two conclusions may be drawn on the nature of these so-called "protective ferments":

1. That there is no evidence of the production of true and specific ferments in pregnancy and disease in the meaning of Abderhalden. On the other hand, there may be an increase of such leukocytic and serum ferments as the proteases in at least some pathologic conditions.

¹ Jour. Biol. Chem., 1915, 22, 59; *ibid.*, 1916, 25, 163.

² Amer. Jour. Physiol., 1915, 39, 1; *ibid.*, 1916, 42, 558.

³ Jour. Exper. Med., 1915, 21, 211.

⁴ Jour. Infect. Dis., 1914, 14, 351.

⁵ Münch. med. Wchn., 1914, 801.

⁶ Münch. med. Wchn., 1914, 1167.

⁷ Compt. rend. Soc. de biol., 1914, 162.

2. That in pregnancy and certain diseases there may be produced more or less specific amboceptor-like antibodies demonstrable by Abderhalden's methods, but not by ordinary chemical methods.

Mechanism of the Abderhalden Reaction; Bronfenbrenner's Theory.—Aside from the probable clinical value of the methods devised by Abderhalden in the serum diagnosis of pregnancy and various pathologic conditions, as malignancy, tuberculosis, lesions of the nervous system and ductless glands, most interest concerns the question of the specificity of the "ferments" or antibodies concerned and the mechanism of their action.

While Abderhalden and many of his pupils have claimed a high degree of specificity for the "protective ferments" and his pregnancy reaction, claiming from the beginning that errors of technic were largely responsible for the failure of others to obtain satisfactory results, the dialysis test as now conducted is not especially difficult, and sufficient work has been done by other investigators who have followed Abderhalden's technic with great care and exactness to give warrant to the claim that other factors aside from those purely technical may be responsible for the divergent and non-specific results obtained.

As previously stated, Abderhalden bases his theory concerning the "protective ferments" upon the specific digestion of a substrat by specific ferments, claiming that these ferments are separate and distinct antibodies, and not to be classed with the cytolytic amboceptors or cytolsins of Ehrlich.

That the substrat in the pregnancy test is a boiled tissue would seem to impair the specificity of the reaction and, indeed, certain physical factors, as the mechanical state of division of the substrat and its facility for acting as an absorbent in a purely mechanical capacity, likewise appear to be factors in the reaction on the basis of numerous investigations, showing that loose areolar placental tissue is frequently digested by normal sera and various pathologic sera irrespective of pregnancy, whereas digestion of a firm and compact tissue as that of malignant tumors is much less constant. In this connection the work of de Waele¹ has a bearing, inasmuch as he found that any agent which would cause an alteration of the physical state of the serum globulins would cause an intense Abderhalden reaction, concluding that the reaction depended upon a globulinolysis having an origin in physical processes probably analogous to the precipitin reaction.

While immunologic as well as chemical and physical reactions are more or less dependent upon quantitative factors, investigations by Flatow² and Herzfeld,³ Plaut,⁴ and others show that while specific results may be obtained by proper manipulation of the material, non-specific results in either a negative or positive reaction may be obtained with practically any serum, however well controlled, with the same material. These investigations are significant not so much because of quantitative factors alone as they are by reason of indicating that pregnancy reaction is dependent upon the principles of mechanical absorption on the part of the substrata of something from the serum followed by a digestive process, rather than upon the simple digestion of a specific substrata by a specific ferment.

For this conception of the mechanism of the Abderhalden reaction the investigations of Jobling and Peterson⁵ discussed above have been fundamental and of great interest and importance. As previously stated, they

¹ Ztschr. f. Immunitätsf., orig., 1914, 12, 170.

² Münch. med. Wchnschr., 1914, Ixi, 468; *ibid.*, 608; *ibid.*, 1168.

³ Biochem. Ztschr., 1914, lxi, 103.

⁴ Münch. med. Wchnschr., 1914, Ixi, 238.

⁵ Jour. Exper. Med., 1914, xix, 459 and 480.

have shown that the digestive power of a serum is dependent upon non-specific proteolytic ferments or proteases normally present and held in check by an antiferment which, according to their work, is believed to reside in the unsaturated fatty acids of the serum. Upon removal of the antiferment by means of lipoidal solvents or saturation with various organic and inorganic substances, as boiled tissue, iodine, starch, kaolin, and the like, protease activity is released, followed by digestion, not of the so-called substrata but of the protein of the serum. Likewise, Plaut,¹ Peiper,² Friedman and Schonfield,³ and Bronfenbrenner⁴ have obtained positive Abderhalden reactions with guinea-pig and human sera, not only with placental tissue but also with such inert substances as kaolin, starch, barium sulphate, chloroform, etc. These studies would, therefore, tend to show that the boiled placental tissue in Abderhalden's reaction is not digested, but acts simply as an absorbent in a purely mechanical manner.

Heilner and Petri⁵ and de Waele⁶ found the ferments in the blood-serum so quickly after the parenteral introduction of the protein, at intervals hardly sufficient for the elaboration of new and specific ferments, as to support the theory that the ferments are preformed and that the substrata serves to activate these rather than bring about the production of new ferments.

It would appear, therefore, that the original theory of Abderhalden is untenable in that specific proteolytic ferments in the blood are not produced during pregnancy, and that the Abderhalden reaction is not due to the digestion *in vitro* of specific antigen by specific ferments.

As indicated by the researches mentioned above and especially the work of Bronfenbrenner, it is now generally accepted that the placental tissue employed in Abderhalden's test simply removes the antiferment of the serum which is followed by the digestion of the serum proteins rather than of the placental tissue, by the non-specific proteases of the serum. During pregnancy, the infectious diseases, in cancer, cachexia, and other diseased states there may be an increase of proteolytic ferments of the serum, but these ferments are non-specific.

The question then arises why the Abderhalden test properly conducted with placental tissue yields more ninhydrin reacting substances than when the test is conducted with kaolin, charcoal, or some other tissue? In so far as my own experiments with Williams⁷ are concerned, I have invariably found that the reactions in pregnancy were stronger when the tests were conducted with pregnant serum and placental tissue. A careful study of the Abderhalden dialysis reaction in Wells' laboratory by Elsesser,⁸ using Osborne's purified vegetable proteins, showed that in spite of many atypical, irregular, and illogical results, "there is an obvious tendency for a substrata to react more often and yield stronger reactions when tested against its homologous immune serum, than when tested against a heterologous immune serum."

The investigations of Bronfenbrenner⁹ apparently offer a satisfactory explanation. As previously stated his studies indicate, but do not definitely prove, that the so-called "defensive ferments" are antibodies of the ambo-

¹ Münch. med. Wchnschr., 1914, lxi, 238.

² Deut. med. Wchnschr., 1914, xl, 1467.

³ Berl. klin. Wchnschr., 1914, li, 348.

⁴ Proc. Soc. Exper. Biol. and Med., 1914, xi, 90.

⁵ Münch. med. Wchnschr., 1911, lx, 1530.

⁶ Ztschr. f. Immunitätsf., orig., 1914, xxii, 31.

⁷ Amer. Jour. Obstet., 1915, lxxii, No. 1.

⁸ Jour. Lab. and Clin. Med., 1915, 1, 79.

⁹ Jour. Infect. Dis., 1916, 19, 655.

ceptor class. These antibodies contained in the serum are believed to sensitize the placental tissue antigen just as hemolytic amboceptors sensitize homologous corpuscles. The sensitized placental antigen then serves to remove the antiferment of the serum which releases the non-specific proteolytic ferments followed by autodigestion of the serum rather than of placental tissue.

According to this theory the process is both non-specific and specific—non-specific in that the plain placental tissue may absorb some antiferment and partially release proteolytic ferments of the serum, and specific in that the antibody of a pregnant serum sensitizes the placental antigen and this sensitized antigen removes antiferment by inducing a change of the colloids of the serum followed by autodigestion of the serum, and the production of ninhydrin reacting substances. According to Bronfenbrenner, the sensitized antigen will remove antiferment from any serum, pregnant or non-pregnant female, or, male serum, followed by autodigestion; on the bases of this theory he has devised a modification of the Abderhalden test to be described later.

ANTITRYPSIN TEST

The antitryptic activity of blood sera has been found to vary in pathologic conditions and tests for measuring variation in antitryptic activity have been advocated as diagnostic procedures.

Brieger¹ originally asserted that 95 per cent. of cases of cancer evinced a marked increase in the antitryptic activity of the serum. He subsequently found that in a large number of other conditions, including both acute and chronic wasting diseases accompanied by cachexia, similar changes may occur. Further investigations have confirmed these findings indicating that the change in the serum is not to be regarded as characteristic of new growths, as it occurs in too many other pathologic conditions and even in physiologic conditions, to have the value of a specific symptom. On the other hand, the absence of the antitryptic reaction in the blood may be taken generally as evidence against the existence of cancer. Weil² has reviewed the literature on this subject and given the results of his own experiences which are largely in accord with the above statements.

Various methods for measuring antitryptic activity of sera have been described, those of Bergmann and Meyer,³ Müller and Jochmann,⁴ Fuld and Goss⁵ being best known. Of these tests that of Fuld and Goss is regarded best, the technic being as follows:

Solution of Trypsin.—This is made by dissolving 0.5 gm. of pure trypsin (Grübler) in 50 c.c. of NaCl solution and adding 0.5 c.c. of normal soda solution; make up to 500 c.c. with physiologic salt solution.

Casein Solution.—Dissolve 1 gm. of pure casein in 100 c.c. of decinormal sodium hydroxid solution with the aid of gentle heat. Neutralize to litmus with $n/10$ hydrochloric acid solution and dilute with physiologic salt solution up to 500 c.c. Filter and sterilize in an Arnold sterilizer. Preserve in the refrigerator.

Acetic Acid Solution.—To 5 c.c. of acetic acid (c. p.) add 45 c.c. of absolute alcohol and 50 c.c. of distilled water.

The **patient's serum** must be fresh, and should be diluted twenty times with salt solution. Dose, 0.2 c.c.

Technic.—A titration of the trypsin solution must precede the test proper.

¹ Berl. klin. Wchn., 1908, 1349 and 2260.

² Amer. Jour. Med. Sci., 1910, 139, 714.

³ Berl. klin. Wchn., 1908, No. 37.

⁴ Münch. med. Wchn., 1909, Nos. 29 and 31.

⁵ Archiv. f. exp. Path., 1907, 137.

Into each of several small test-tubes place increasing amounts of trypsin solution, as, for example, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 c.c. Add 2 c.c. of the casein solution to each tube; shake carefully and place in an incubator or water-bath for half an hour at 50° C. Then add 3 or 4 drops of the acetic acid solution to each tube, and observe which tube first shows cloudiness after a few minutes. The tube containing the smallest amount of trypsin and which remains perfectly clear contains enough trypsin fully to digest the 2 c.c. of casein solution.

Into each of six small test-tubes now place 0.2 c.c. of the 1 : 20 dilution of the patient's serum, and increasing amounts of the trypsin solution, beginning with the completely digesting dose, as determined above, and increasing by 0.1 c.c. Add 2 c.c. of casein solution to each tube, and bring all tubes to a like volume by the addition of normal salt solution. Shake gently and incubate at 50° C. for half an hour. Add several drops of acetic acid solution to each tube, and again observe the tube containing the smallest amount of trypsin in which cloudiness can be seen. Thus the amount of trypsin neutralized by the antitrypsin of the serum is determined.

For example, in an experiment the preliminary titration showed that 0.5 c.c. of trypsin completely digested the casein. In the second part of the test the lower limit of trypsin was this 0.5 c.c. increased by 0.1 c.c. in successive tubes up to 1 c.c. It is now found that 1 c.c. of the trypsin solution is required to bring about the complete digestion of the casein in the presence of the serum, or 1 c.c. — 0.5 c.c. = 0.5 c.c., which is the amount of trypsin neutralized by 0.01 c.c. of undiluted serum.

A control experiment is conducted with the pooled serum of several normal persons, and a comparison of the value thus obtained shows whether the antitryptic power of the serum tested is altered.

Robertson and Hanson¹ have recently described a simple, accurate modification of this method for measuring the antitryptic indices of blood-sera.

The method of Marcus,² which is a modification of the method of Müller and Jochmann, is described in the laboratory exercises on Experimental Infection and Immunity. This technic, however, is not as accurate as that of Fuld and Goss, described above. Both egg-albumens and the coagulated sera of different animals vary considerably in digestibility, so that there is not the required constant basis of comparison. Furthermore, the visual appreciation of a minute depression on the surface of a serum plate is a very difficult and inexact procedure.

ABDERHALDEN'S TESTS³

Methods.—Two methods have been devised by Abderhalden for the demonstration of antibody or the "ferments" in the blood-serum of pregnancy, cancer, and other conditions:

1. *The Dialyzation Method.*—Specially prepared and coagulated placenta and fresh serum are placed in a dialyzing capsule so prepared that it will permit the passage of peptones and amino-acids only. The filled capsule is placed in sterile distilled water, and incubated for from sixteen to twenty-

¹ Jour. Immunology, 1918, 3, 131.

² Berl. klin. Woch., 1908, No. 4; 1909.

³ Abderhalden: Abwehrfermente des tierischen Organismus, Julius Springer, 3d ed., 1913. The author realizes that he is open to criticism for devoting space to a description of Abderhalden's methods, since the reaction has been proved to lack reliable diagnostic value in pregnancy at least. On the other hand, I doubt the wisdom of "scrapping" the immense literature (some of which is thoroughly reliable and acceptable) and dropping the subject; I still believe that immunologic changes may occur in the serum in pregnancy, and that, while Abderhalden's theory and methods may be wrong, the subject is worthy of more investigation. It is for this reason that one of his methods is described for the aid that may be given students of the subject.

four hours, when the dialysate is tested by the biuret or ninhydrin test for peptones and amino-acids. Under proper conditions the presence of these substances indicates a positive reaction.

2. *The Optical Method.*—This method is based upon the same principle as the dialyzation method. Into the tube of a polariscope place a solution of placental peptone and the serum to be tested. Warm the mixture to 37° C., and after an hour note the degree of rotation and record it; repeat this at intervals during the following twenty-four to forty-eight hours. If the serum contains the antibody or "ferment," the peptone is split into amino-acids and the degree of rotation increased from 0.05° to 0.5° C. and higher. This method requires an expensive polariscope, considerable practice in making the observations and readings, and is only reliable in skilful hands.



FIG. 93.—A DIALYZING CYLINDER FOR THE ABDERHALDEN FERMENT TEST.

The shell contains placental tissue and fresh serum; it is surrounded with 20 c.c. of distilled water and covered with toluol. The cotton plug prevents contamination. The cylinder is readily sterilized in a hot-air oven and affords a simple and efficient means for conducting the test by the dialyzation method.

THE DIALYZATION METHOD

Testing the Dialyzing Shell.—The quality of the dialyzing shell largely determines the success of this method. It must fulfil two requirements:

1. It must be absolutely non-permeable for albumin.
2. It must be evenly permeable for the protein cleavage products, such as peptones, polypeptids, and amino-acids.

Special shells are made by Schleichter and Schull, No. 579a being recommended at the present time. The shells must be of correct size, and every one must be tested before being used. If a shell allows uncleaved protein to pass through, then all reactions would react positively regardless of the presence or the absence of the specific ferment. If the shell is too thick and too tight, and prevents the passage of peptones and amino-acids, then all reactions would be negative, even though the ferment were present in the serum and had digested the placental protein. *Accordingly, each shell must be tested and standardized, and only those employed that have proved satisfactory.*

Glassware.—It is highly important that all glassware should be free from clinging particles or traces of albumin, acids, and alkalis. Pipets and dialyzing cylinders should be washed in water, alcohol, ether, and finally in distilled water, and sterilized by dry heat. Boiling rods of solid glass (10 by 0.5 cm.) should be washed in alcohol, ether, and distilled water, wrapped in bundles of six in newspaper, and sterilized by dry heat.

A very convenient dialyzing cylinder is shown in the accompanying illustration (Fig. 93). This cylinder measures 8 by 3 cm. It should be plugged with cotton and sterilized. When the shell is loaded with coagulated placenta and serum and covered with toluol, it will rest well beneath



FIG. 94.—NINHYDRIN REACTION (ABDERHALDEN FERMENT TEST).

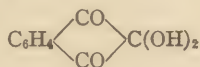
The tube on the left shows a positive reaction with the serum of a pregnant woman; the tube on the right is the serum control and shows a faint violet color, due, presumably, to the passage of dialyzable substances in this serum.

the surface of the outside distilled water. The wide mouth of the cylinder facilitates all manipulations and the shell cannot upset. The apparatus is easily sterilized, and the cotton plug prevents bacterial contamination and undue evaporation of the contents.

General Precautions.—According to Abderhalden, the work should be conducted in a special room, where there is no dust or fumes of acids, and where no bacteriologic work is in progress. This observer also recommends that a special incubator be used for this work. If, however, the working table is scrupulously clean and the glassware is clean and sterile, and if the shells are handled with sterile forceps and the dialyzing cylinder is stoppered with a plug of sterile cotton, all requirements are practically fulfilled.

Reagents.—The presence of albumin or its split products may be detected by two color reactions: (1) the biuret reaction, and (2) the ninhydrin reaction. The first is especially delicate for uncleaved albumin, and the latter for peptones and amino-acids. The technic of the biuret test is described with the technic of testing shells for permeability to albumins.

Ninhydrin.—This is the trade name for triketohydrindenhydrate. It is a whitish yellow, readily soluble powder, dispensed in brown glass vials containing 0.1 gm. of the drug. A circular describing its method of use accompanies each package. As 0.2 c.c. of a 1 per cent. watery solution is the amount necessary for a test, the contents of the vial are dissolved in 10 c.c. of distilled water, and the vial rinsed with a portion of the solvent. This solution should be preserved in a brown bottle in a cold place, and precautions taken to prevent infection. Triketohydrindenhydrate has been described by Ruheman,¹ who gives its formula as follows:



Owing to the fact that it gives a blue color in the presence of any compound that possesses an amino-group in the alpha position of the carboxyl group, it is of great value as an aid in recognizing the products of protein digestion (Fig. 94).

Testing the Shell for Non-permeability to Albumin.—1. New shells should be softened by soaking them for half an hour in sterile distilled water. A dozen or more may be tested at one time.

2. The albumin solution is prepared by placing 5 c.c. of the albumin of fresh eggs in a mixing cylinder, and adding distilled water to make 100 c.c. Mix well. There must be no flakes. Instead, a clear, hemoglobin-free serum which has been dialyzed against running water to remove dialyzable substances, may be used in doses of 2.5 c.c. for each shell.

3. Carefully pipet 5 c.c. of the albumin solution into each shell. Great care should be exercised that none of the solution contaminates the outside of the shell. The preferable method is to hold the shell with a pair of broad-toothed sterilized forceps and carry the pipet to the bottom, in order that none of the albumin should contaminate the upper portion of the inside of the shell. The pipet may easily touch the edge of the shell and thus contaminate the dialysate. If in doubt, cover the upper end of the shell with the forceps and wash the outside with running water.

4. The loaded shell is now placed in a sterile dialyzing cylinder containing 20 c.c. of sterile distilled water. *Never load the shell in this cylinder,* for some of the albumin may fall into the distilled water.

5. Cover the contents of the shell and the surrounding distilled water

¹ Jour. Chem. Soc., London, 1910, xcvi, 2025.

with a layer of toluol about $\frac{1}{4}$ inch in depth. Replace the cotton plug in the cylinder.

6. Incubate at 37° C. for sixteen hours.

7. Pass a sterile pipet quickly through the layer of toluol and remove 10 c.c. of the dialysate to a clean sterile test-tube, and test for albumin by the *biuret reaction*. Add 2.5 c.c. of a 33 per cent. solution of sodium hydroxid; shake gently, but remove the thumb from the top of the tube. The solution may become slightly cloudy. Carefully overlay with 1 c.c. of a 0.2 per cent. solution of copper sulphate in such manner that a sharp line of demarcation separates the alkaline dialysate from the copper sulphate solution. A delicate violet tint at this line indicates that albumin is present, and that the shell is useless. If one cannot see this color or is in doubt, it is well to make the ninhydrin test. To do this dialysis should be continued for twenty-four hours; ninhydrin reacts with albumin in addition to peptones and amino-acids, but according to Abderhalden, this test is less sensitive than the biuret test.

8. All shells should react negatively, *i. e.*, they should not permit the passage of unchanged albumin. If the ninhydrin test is used, the tubes should be inspected one-half hour after boiling, and the contents should be as clear as water or show but the faintest blue tint. If this is not the case, shells should be discarded as being permeable to albumin. Those that are satisfactory in this respect should be tested further as follows:

Testing the Shell for Permeability to Peptone.—1. The shells should now be thoroughly cleansed, but not with a stiff brush, washed in running water, and boiled for thirty seconds.

2. Prepare a 1 per cent. solution of silk peptone (Höchst) in distilled water, and carefully pipet 2.5 c.c. into each shell, using every precaution against contaminating the upper portion on the inside, and especially of the outside, of the shell.

3. Place the loaded shell in a sterile dialyzing cylinder containing 20 c.c. of sterile distilled water, and cover the contents of the shell and water with toluol. Replace the cotton plug and incubate at 37° C. for twenty-four hours.

4. Remove 10 c.c. of the dialysate (avoid removing toluol) to a clean, sterile, *thin-walled* test-tube, and add 0.2 c.c. of the 1 per cent. ninhydrin solution. Insert a sterile boiling rod and *boil for exactly one minute*.

5. The boiling process is quite an important feature of this test. Always boil in precisely the same manner. A high Bunsen flame should be used, and about one minute after air-bubbles first appear on the sides of the tube lively boiling commences. The flame should then be turned down and the boiling continued for exactly one minute.

6. Place the tube in a rack. With a fresh sterile pipet remove 10 c.c. of dialysate from the next cylinder and test in the same manner, and repeat until the entire series have been finished.

7. After half an hour inspect all the tubes; they should show a deep blue color; if they do not do so they are impermeable or partly permeable to peptone and should be discarded. There is usually a difference in the degree of color reaction among a number of shells, as their permeability varies.

8. Those shells that have withstood both tests are now thoroughly washed in running water, boiled for thirty seconds, placed in a jar of sterile distilled water containing a few drops of chloroform, and covered with toluol. From this time on they should not be handled with the fingers, but only with forceps that have been sterilized by boiling. Of the entire

number of shells, usually from 20 to 30 per cent. or more are found to be unsatisfactory.

Preparation of the Placental Tissue.—This is the substratum, and should consist of coagulated placental protein free from dialyzable substances that react with ninhydrin.

1. A fresh normal placenta should be prepared soon after delivery. It is *highly important to wash it free from all blood*, Abderhalden having laid considerable stress upon this point. He explains that in the blood of all animals there is always a specific ferment for the red blood-corpuscles, as even the smallest hemorrhage into the tissue calls forth a protective ferment. For this reason all organs that contain blood may contain the substratum and ferment, and yield false positive reactions.

2. The placenta should be placed in warm water and freed as far as possible of clots. The membranes and cord are removed, and the placental tissue cut into pieces about the size of a dime. These are placed in a sieve under running water, and each piece squeezed with the hand. From time to time the entire mass is thoroughly squeezed out in a towel. Tissues that cannot be freed from clots should be discarded. The tissues are now crushed in a mortar, connective-tissue strands removed, and the washing continued until the tissue is *snow white*. Decolorizing substances, such as H_2O_2 , should not be used. If the tissue is not white and free from blood it should not be employed. Liver, spleen, and kidney tissue cannot be made perfectly white, although all traces of blood have been removed.

3. Place 100 times as much distilled water as there is tissue in an enameled vessel; to each liter add 5 drops of glacial acetic acid and heat to boiling when the tissue is added and boiled for ten minutes.

4. Wash the coagulated tissue with distilled water, and boil again without the addition of acid. This should be repeated six times in succession. If an interruption occurs, cover the tissue and water with a layer of toluol.

5. After the sixth boiling add a small quantity of water to the tissues—just sufficient to enable it to boil for about five minutes without burning, for the water is now to be tested with the ninhydrin reaction and it is important that this be as concentrated as possible. Filter the water, and to 5 c.c. in a sterile test-tube add 1 c.c. of the ninhydrin solution. Boil vigorously for one minute. If there is the slightest discoloration within half an hour, the tissues must be boiled again, but with only five volumes of water and no longer than five minutes each time. These boilings should be repeated as often as is necessary until the *ninhydrin reaction remains water clear for at least one-half hour*.

6. The tissues are again gone over with a sterile forceps, and a search made for brown masses resembling blood-clots. These are to be discarded.

7. The tissue is now preserved in a sterile jar containing sufficient sterile water and chloroform and covered with toluol. All tissue should be handled with sterile forceps, and when once removed from the jar, they should never be returned. The whole operation requires several hours and it should be conducted without interruption. If the process is interrupted, the tissue should be covered with a layer of toluol.

8. It is well to try out the tissue with a known serum of pregnancy to make certain that it is a suitable substratum.

9. Only normal placenta should be used, as in certain instances a normal organ may be satisfactory, whereas a diseased organ would be unsuitable.

10. Animal placenta may be substituted for human placenta and vice versa, but Abderhalden cautions against this substitution until further work has been done.

The Blood-serum.—The serum to be tested must fulfil three conditions:

(1) It must contain the smallest amount of dialyzable substances that would react with ninhydrin. Blood is best drawn in the morning before breakfast. In all diseases accompanied by marked protein disintegration, such as cancer, the blood-serum may contain large amounts of dialyzable substances.

(2) It must be *absolutely free from hemoglobin* and clear.

(3) It must be *free from cells*. Even an apparently clear serum may contain millions of erythrocytes.

1. From 10 to 20 c.c. of blood are withdrawn from a vein at the elbow with a dry sterile needle into a sterile centrifuge tube. This is placed aside at room temperature for several hours, when sufficient serum has usually separated out; if this has not occurred, centrifuge for several minutes. The serum is removed to a second sterile centrifuge tube, and centrifuged at high speed for several minutes until all corpuscles have been precipitated to the bottom of the tube.

2. The serum should be used within twelve hours after the blood has been withdrawn. Abderhalden claims that heating a serum to 60° C. robs it of its digesting powers. Pearce and Williams have found that inactivation considerably weakens the reaction, but does not abolish it altogether.

3. Specimens of blood sent through the mails are really unsatisfactory for even if they are delivered within twelve hours after bleeding the amount of handling has usually resulted in the breaking up of a number of corpuscles, and the tingeing of the serum with hemoglobin.

The Test.—1. Absolute cleanliness should be employed. The glass-ware should be sterile and dry, and everything should be in readiness. The technic should be aseptic and thoroughly understood.

2. Remove a sufficient amount of the prepared placenta for the work at hand with sterile forceps and wash in a dish of sterile distilled water to remove toluol and chloroform. Boil with 4 or 5 volumes of sterile distilled water for two minutes and test the water with ninhydrin. If positive, the tissue must be boiled as described above until free of ninhydrin reacting substances. Place on sterile filter-paper and squeeze to remove any excess of water. Weigh and place 0.5 gm. in each of two shells (one for a control).

3. Holding each shell with a second pair of boiled forceps, pipet 1.5 c.c. of the patient's serum into one shell containing placenta, and the same amount into a third shell which is to serve as a control on the serum. Place 1.5 c.c. of sterile distilled water in the placental tissue control shell.

4. Unless one is absolutely sure that neither the tissue nor the serum has touched the outside of the shells they should be held shut with sterile forceps and washed with sterile distilled water.

5. Each of the three shells is now placed in cylinders containing 20 c.c. of sterile distilled water. *Under no circumstances are the shells to be loaded while they are in the dialyzing cylinders.*

6. The contents of each shell and the water surrounding them are covered with a layer of toluol about $\frac{1}{4}$ inch in depth, and the cylinders plugged with cotton to prevent evaporation and contamination. The shell should be at least $\frac{1}{4}$ to $\frac{1}{2}$ inch above the level of the outside fluids, and due care must be exercised in carrying the cylinder back and forth from the incubator that the contents of the shell and the surrounding water do not become mixed.

7. If it is at all possible, it is well to set up two more shells as controls, each containing placenta and normal serum and the serum of pregnancy respectively.

8. All the cylinders are incubated at 37° C. for twenty-four hours. Ten c.c. of the dialysate are then removed from each tube with a separate sterile pipet and placed in sterile test-tubes of the same size and boiled with 0.2 c.c. of the 1 per cent. ninhydrin solution for exactly one minute. After standing for half an hour the readings are made.

Reading the Reaction.—The dialysate of the serum alone should be clear as water or show but the faintest blue tinge. The dialysate of the placenta alone should be clear; the dialysate of the patient's serum plus that of the placenta may show a deep violet-blue color when the reaction is strongly positive, or a fainter blue when it is weakly positive. If this dialysate is water clear or has a faint blue color, comparable to the controls, the result is negative. If there is any doubt the test should be repeated. The negative control should be water clear or have a faint tinge comparable to its control. The positive control should show a deep violet-blue color.

I generally control the result given by the shell containing tissue and patient's serum by cleansing it thoroughly, boiling for a minute, and testing it with egg-albumen solution or a serum in case the reaction was positive, to make sure that the shell has not allowed the passage of serum, or with peptone solution in case the reaction was negative, to make sure that it was not thick enough to block the passage of peptones and amino-acids. This procedure delays the report on a serum for another twenty-four hours, but the greater accuracy obtained warrants the delay.

Readings should never be made by artificial light. Tubes should be held against a white background the better to appreciate the color changes.

A pinkish- or brownish-yellow discoloration has nothing to do with the ninhydrin reaction.

Sources of Error in the Dialyzation Method.—There are many sources of error, and until the technic has been improved sufficiently to eliminate these, Abderhalden's directions should be followed minutely.

1. The shells may become spoiled in time. They should not be cleansed with rough brushes or boiled too long. They should be cleansed at once after using, and tested every four weeks. If a wrong diagnosis results the shell should be retested at once.

2. The placental tissue is an important source of error, due to the fact that it contains blood.

3. The serum should be fresh and free from hemoglobin and corpuscles.

4. The controls on placenta alone and each serum alone are absolutely necessary, as both may contain various substances capable of reacting with ninhydrin and thus yielding false positive reactions.

5. The water used should be distilled and sterile. The glassware should be chemically clean and sterile, and the laboratory free from the fumes of acids and alkalis. It is very important that absolutely the same conditions should exist for the control tests as for the main test itself.

Bronfenbrenner's Modification.—Bronfenbrenner, Schlesinger, and Mitchell¹ have sought to improve the test described above by some modification in technic embracing the principle that serum may contain an antibody-like substance capable of sensitizing the antigen, and that this sensitized antigen removes antiferment by producing a change in the colloids of the serum, followed by autodigestion (of the serum), and the appearance of ninhydrin reacting substances. The test may be conducted as follows:

1. Serum is collected and placental tissue or other antigen are prepared as described above.

2. Fresh serum (1.5 c.c.) and 0.5 gm. of antigen are placed in a sterile

¹ Jour. Amer. Med. Assoc., 1915, 65, 1268.

centrifuge tube, stoppered, and kept on ice for sixteen to eighteen hours. A control with normal serum should be set up in the same manner. During this time the tissue antigen is being sensitized by the antibody in the pregnant serum and the sensitized antigen in turn removes the antiferment. At the same time the antigen in both pregnant and normal control serum is probably absorbing some antiferment in a non-specific manner. At this low temperature autodigestion of serum does not occur.

3. The tubes are now thoroughly centrifuged and each serum transferred to a thimble prepared as described. Dialyzation is now conducted in a thermostat for twenty-four hours as in the Åbderhalden test, and the dialysate tested with ninhydrin as described, which completes the test. The dialysate from the thimble carrying exhausted pregnancy serum yields a positive ninhydrin reaction due to autodigestion by reason of specific removal of antiferment by sensitized antigen in the preceding phase; the dialysate from the thimble carrying normal serum yields a negative ninhydrin reaction, or a weak reaction due to the partial removal of antiferment by non-specific absorption in the preceding phase.

In addition, the tissue antigen may be washed twice with saline solution by centrifuging and, after the last washing, transferred to a thimble with 1.5 c.c. of a fresh normal serum (preferably the serum of a male guinea-pig unfed for at least eight hours previous to bleeding). Dialyzation is conducted as described and the dialysate tested with ninhydrin. Sensitized antigen (placental tissue sensitized with pregnant serum) produces ninhydrin reacting substances in the dialysate by removal of antiferment, followed by autodigestion of the serum; plain antigen produces much less or no ninhydrin reacting substances.

PRACTICAL VALUE OF ÅBDERHALDEN'S TESTS

Pregnancy.—An enormous literature has accumulated on the application of Åbderhalden's methods in the diagnosis of pregnancy. Some of the reports by competent investigators are favorable to its practical value; others are unfavorable, while the majority report a high percentage of positive reactions with the sera in pregnancy along with non-specific results with the sera of non-pregnant persons and lower animals. In general, it may be stated that the Åbderhalden test has failed as a practical diagnostic procedure and has been largely discarded; the practical value of Bronfenbrenner's modification awaits to be determined.

Some authors have summarily dismissed the whole subject, but I am not able to do this and believe that it deserves the discussion devoted to it in the preceding pages. That Åbderhalden is wrong in his conception of the nature of the so-called "protective ferments" and the mechanism of their action may be regarded as proved; on the other hand, I am convinced that in pregnancy and other conditions there may be found in the serum a specific antibody-like substance in addition to an increase of non-specific proteolytic ferments.

The very technic developed by Åbderhalden may not serve for the differentiation of sera containing the specific antibody-like substances from those that do not but, nevertheless, I believe that enough work has been done to show that a specific substance may be present worthy of continued efforts in the way of improvement in technic for its detection. After the technic has been satisfactorily developed, and especially along the lines of Bronfenbrenner's method, I believe that it is worth while to again study the sera in pregnancy from the standpoint of a practical test.

In addition to pregnancy the general results of a large number of investigations indicate that similar antibody-like substances may be found in the serum in cancer, degenerative lesions of the central nervous system, and various bacterial infections. I have briefly mentioned in the following paragraphs some of these investigations, not because the present methods are generally acceptable for the detection of this antibody-like substance from the standpoint of practical tests, but to indicate that the antibody may be found in many different pathologic conditions.

Cancer.—A large literature has also accumulated on the results of Abderhalden's tests in cancer; Freund and Abderhalden¹ claim to have found the "ferments" in the serum of cancer that will yield positive reactions with cancer tissue. Frank and Heiman² reported positive results in 53 of 54 cases of cancer; Markins and Munze,³ Epstein,⁴ Gambaroff,⁵ Erpicum,⁶ Brockman,⁷ Lampe,⁸ Lowy,⁹ Ball,¹⁰ and others have reported highly favorable results. Frankle¹¹ and Lindig¹² have found the reactions generally non-specific in character.

It is well to make the tests with a number of cancer tissues taken from various parts, also with sarcoma tissue and that of various benign tumors.

Mental Diseases.—Fauser¹³ has studied the serums of 88 cases of dementia præcox and other mental diseases with various antigens composed of the ductless glands, testicles, ovaries, etc., and attained interesting results, tending to show that in many of these brain affections there may be associated lesions in other organs, and that the symptoms may be due to perverted functions of certain ductless glands. Munzer,¹⁴ Bundschue and Roener,¹⁵ and Fisher¹⁶ have also found in the serums of mental and nervous diseases "ferments" reacting with the tissues of the ductless and generative glands, tending to show that lesions of these organs may be operative in the symptomatology of these conditions.

Syphilis.—Baeslack¹⁷ has reported having had exceptionally good results with the serums of syphilitics and a substratum composed of coagulated syphilitic lesions of rabbit's testicle. Using the dialyzation method he found the sero-enzyme test more constant and earlier than the Wassermann reaction.

Tuberculosis and Acute Infections.—Abderhalden and Andryewsky¹⁸ have suggested the use of the dialyzation or the optic method in the diagnosis of acute infections. The peptone may either be prepared of the bacilli, or the boiled organisms used in the dialyzing shell. In preparing a bacterial substratum the material must be carefully centrifuged in order to facilitate washing. The tubercle bacilli are degreased by extraction in fat solvents.

¹ Münch. med. Wchnschr., 1913, 14, 763.

² Berl. klin. Wchnschr., 1913, 1, No. 14.

³ Berl. klin. Wchnschr., 1913, 1, No. 17.

⁴ Wien. klin. Wchnschr., 1913, xxvi, No. 17.

⁵ Berl. klin. Wchnschr., 1913, No. 17.

⁶ Bull. de l'Acad. Roy. de Belg., 1913, xxvii, 624.

⁷ Lancet, London, November 15, 1913.

⁸ Münch. med. Wchnschr., 1914, lxi, No. 9.

⁹ Jour. Amer. Med. Assoc., 1914, lxii, 437.

¹⁰ Jour. Amer. Med. Assoc., 1914, lxii, 599.

¹¹ Deutsch. med. Wchnschr., xl, No. 12.

¹² Münch. med. Wchnschr., 1913, 60, 288.

¹³ Deutsch. med. Wchnschr., 1913, xxxix, No. 7.

¹⁴ Berl. klin. Wchnschr., 1913, 1, No. 5.

¹⁵ Deutsch. med. Wchnschr., 1913, No. 42, 2069.

¹⁶ Deutsch. med. Wchnschr., 1913, No. 44, 2138.

¹⁷ Jour. Amer. Med. Assoc., 1914, lxii, 1002; *ibid.*, lxiii, 559.

¹⁸ Münch. med. Wchnschr., 1913, lxi, 1641.

Abderhalden and Andryewsky found "ferments" present in the serum of cattle receiving injections of suspensions of dead tubercle bacilli and in experimental infections, and suggest that the test may prove efficacious in testing cattle. This work should receive further study in human infections. Smith,¹ employing Bronfenbrenner's modification of the Abderhalden test, has reported highly specific results in the differentiation of various bacteria with rabbit immune sera.

THE TWORT-D'HERELLE PHENOMENON OF BACTERIOLYSIS.

While various enzymes of different sources may digest dead micro-organisms and play a part in resistance to infection and recovery from disease, the bacteriolytic agent discovered by Twort and d'Herelle is capable of dissolving *living* bacteria of various kinds, and its discovery constitutes not only an important advance in our knowledge of bacteriology, but likewise possesses great interest in relation to the processes of infection and immunity. I have already referred to the subject in previous chapters in relation to infection and immunity, and wish at this place to discuss more completely the source, nature, properties, and significance of this newly discovered bacteriolytic agent, which some investigators regard as a bacterial enzyme.

In 1915 Twort² observed in cultures of micrococci prepared by plating glycerinated cowpox vaccine certain transparent or degenerated areas in which the cocci did not grow. When material from these areas was streaked through a culture of the micrococci it was observed that the colonies became clear and transparent within a few hours. When material from a transparent area was passed through a Berkfeld filter and the filtrate added to cultures of the micrococcus it was found that the latter were killed and dissolved. In other words, a filter-passing agent was discovered capable of killing and dissolving living staphylococci; the source of this agent was probably the micrococci or cowpox vaccine, but the bacteriolytic agent in the filtrate could be passed on to numerous generations by transferring it to successive fresh cultures of staphylococci and refiltering.

Twort obtained similar results with a bacillus of the colon group, isolated from the intestinal mucosa of a dog with distemper; also with a large bacillus of the colon group from the intestinal discharges of a child suffering from diarrhea. The bacteriolytic agent maintained its activity for six months and was destroyed by heating at 60° C.

This discovery did not attract the attention it deserved due in large part to the absorption of interest by the events and demands of the World War. About the same time d'Herelle³ may be said to have discovered the phenomenon anew and independently by finding in the intestine of locusts a principle antagonistic to the action of a certain pathogenic *cocco-bacillus*. With this suggestive observation as a basis, d'Herelle in 1915 systematically sought for a similar principle in the intestinal contents of patients with intestinal infections, and especially dysentery, which prevailed in a squadron of cavalry stationed in the neighborhood of Paris. It was observed that by inoculating 2 or 3 drops of a stool from a dysentery patient in the Pasteur Hospital (at the outset of clinical improvement) in 20 c.c. of bouillon and

¹ Jour. Infect. Dis., 1916, 18, 14.

² Lancet, London, 1915, 2, 1241.

³ Compt. rend. Acad. d. sc., Paris, 1917, 165, 373; *ibid.*, 1918, 167, 970; *ibid.*, 1919, 168, 631. Also a series of papers in Compt. rend. Soc. de biol., 1918, 1919, 1920, and 1921, volumes 81, 82, 83, and 84. For the latest summary of investigations and discussions consult Smith's translation of d'Herelle's monograph: *The Bacteriophage—Its Rôle in Immunity*, published by Williams & Wilkins Company, Baltimore, 1922.

filtering the culture a few hours later through a Chamberland No. 12 filter, that a trace of this filtrate added to a culture of the Shiga dysentery bacillus was followed by the death and dissolution of the organisms. When this material was filtered and a drop or two of the filtrate added to a fresh culture the phenomenon was repeated.

Since then d'Herelle has studied the subject very extensively, succeeding in discovering a bacteriolytic agent for various bacteria in the feces of human beings and some of the lower animals with various diseases, as well as in the feces of a few normal healthy individuals. He states that the phenomenon was probably first observed by Hankin,¹ who found that the water of the Jumna River below the town of Agra was bactericidal for various micro-organisms and particularly the cholera vibrio; d'Herelle believes that these effects were due to the presence of this filterable bacteriolytic agent.

d'Herelle's Method of Securing the Bacteriolytic Agent.—For isolation from feces a portion of the size of a pea is inoculated into 50 c.c. of ordinary peptone bouillon about -0.6 in reaction to phenolphthalein (lysis will not occur in an acid medium). This culture is incubated at 37° C. for from twelve to eighteen hours, followed by filtration through sterile paper and finally through a sterile earthen filter, as the Chamberland L_2 and L_3 bougies.

The day before the test is to be made an agar slant is inoculated with a strain of dysentery bacillus, and on the day of the test four tubes of broth medium are inoculated from this tube. To the first of these tubes is added 1 drop of the filtrate, to the second, 10 drops, and to the third, 2 c.c. The fourth serves as a culture control.

The tubes are incubated for eighteen to twenty-four hours. If all tubes carrying filtrate show a growth, about 0.02 c.c. from each is spread over the surfaces of three tubes of agar. If, after incubation, these tubes present a normal growth of the dysentery bacillus, the feces did not contain the bacteriolytic agent. If, however, the cultures appear broken up, "moth-eaten," and with areas of no growth, the results are positive.

If, however, one or all three of the broth cultures remain clear while the control shows turbidity, the bacteriolytic agent is present, the amount in the filtrate bearing a relation to the results observed with the different amounts of filtrate. d'Herelle states that the bacteriophage may be present, therefore, without the slightest macroscopic evidence of lysis in the broth culture and that this is usually the case in the process of isolation. In his opinion the bacteriophage multiplies under certain conditions, and he has described methods for enhancing their virulence and for estimating their numerical strength.

Sources and Specificity of the Bacteriolytic Agent.—As stated above, Twort originally discovered the agent in cultures of staphylococci from vaccine lymph; Gratia² has recently confirmed these findings and Bail³ has obtained the substance from Shiga dysentery bacilli. Twort, in addition to his work with staphylococcus cultures, obtained similar results with organisms of the colon-typhoid group and with cultures from cases of canine distemper and of infantile diarrhea.

d'Herelle made daily filtrates of cultures of the stools of 34 cases of dysentery and obtained a bacteriolytic agent as soon as clinical improvement commenced. The filtrates of the stools of patients during the active stage of the disease, of patients that died, or of stools that did not contain Shiga bacilli were not bacteriolytic. He applied the same methods to the

¹ Ann. l'Inst. Pasteur, 1896, 10, 175, 511.

² Proc. Soc. Exper. Biol. and Med., 1921, 18, 192.

³ Wien. klin. Wchn., 1921, 34, 555.

stools of typhoid and paratyphoid patients and obtained bacteriophages active against *Bacillus typhosus* and *B. paratyphosus*. d'Herelle later made cultures from the stools of a healthy man every two weeks for ten months. Contrary to his previous belief that normal stool filtrates had not bacteriolytic power, he obtained from these 23 tests, 2 that were active against *B. shigæ*, 2 active against Flexner bacilli, 1 against paratyphoid A, 3 against paratyphoid B, 3 against *B. coli*, and 1 against hog cholera. Eleven of the 23 stool filtrates were inactive. d'Herelle also obtained a filtrate active against *B. sanguinarum* and another active against *B. gallinarum* from the stools of chickens suffering from avian typhoid. No active filtrates were found in the stools of normal fowls. In cases of human pyelonephritis, of flacherie in silkworms, of hemorrhagic septicemia in cattle, and of plague in rats, d'Herelle isolated bacteriolytic agents.

Bordet and Ciuca¹ introduced a new method for obtaining the bacteriolytic agents. They injected a culture of *Bacillus coli* intraperitoneally into a guinea-pig. The day after the third injection they found that a small quantity of the resulting peritoneal exudate, as well as the culture of *B. coli* isolated from this exudate, would dissolve an eighteen-hour culture of the strain of *B. coli* that had been used for these inoculations. This lysis was not complete and a few colonies could be cultivated. If these surviving organisms were inoculated into another eighteen-hour colon culture, lysis would again result. This bacteriolytic action could in this way be repeated indefinitely. The bacilli exposed to the lytic substance acquired the ability to transfer the lytic property to subsequent generations. Wollstein² repeated this work using a strain of the Shiga bacillus instead of *B. coli*, and obtained an agent bacteriolytic for dysentery and other bacilli.

Dumas³ found filtrates active against *Bacillus shigæ* and *B. coli* in the stools of 5 out of 8 normal individuals who never had had intestinal disease, in the feces of guinea-pigs, in earth, in Paris city water, and in water from the Seine.

Debre and Haguenau,⁴ using d'Herelle's method, found an active bacteriophage in 3 of 6 cases of acute dysentery, in 3 of 16 cases of enteric fever, in 1 case of diarrhea, in 1 case of cancer of the stomach, in 1 case of rheumatic fever, in 1 case of phthisis, and in 2 cases of peritonitis. They were unable to obtain evidence of a bacteriolytic agent in filtrates from the stools of healthy or ill, breast, or bottle-fed infants.

Kuttner⁵ from the stools of a typhoid convalescent obtained a filtrate active against typhoid, Shiga, and Flexner bacilli. She also found that a filtered glycerin extract of the small intestine of a guinea-pig and a saline extract of a guinea-pig's liver were bacteriolytic for typhoid bacilli. This lytic principle could be transmitted by passage through successive typhoid cultures. Glycerin extracts of the large intestine and of muscle tissue were not bacteriolytic.

Wollstein, using d'Herelle's method, made filtrates of the stools of 23 infants including 6 with gastro-intestinal disturbances and 1 convalescent from Shiga dysentery. The stool filtrate of only 1 baby was bacteriolytic. This infant had a fatal *B. coli* peritonitis. The filtrate was active against colon and Shiga bacilli.

¹ Compt. rend. Soc. de biol., 1920, 83, 1293, 1296; *ibid.*, 1921, 84, 276, 278, 280, 745, 747, 748.

² Jour. Exper. Med., 1921, 34, 467.

³ Compt. rend. Soc. de biol., 1920, 83, 1314.

⁴ Compt. rend. Soc. de biol., 1920, 83, 1348.

⁵ Proc. Soc. Exper. Biol. and Med., 1921, 18, 158, 222.

Davison¹ has been able to demonstrate the bacteriolytic agent active against Shiga and Flexner bacilli in filtrates of the stools of a normal infant as well as from those suffering from bacillary dysentery (Flexner). If one- to sixty-day-old broth or peptone water cultures of recently isolated or old laboratory strains of *B. dysenteriae* (Shiga) or (Flexner) were filtered, the filtrate in many instances was slightly bacteriolytic for Shiga and Flexner bacilli. The bacteriolytic agents from the stools of these children and the filtrates of dysentery cultures retained their lytic power after passage through several successive cultures.

From this review it is evident that the filter-passing bacteriolytic agent of Twort and d'Herelle may be secured from various and diverse sources; also that its origin in many instances bears no relation to the type of organism attacked.

While originally believed by both Twort and d'Herelle to be specific, it is now known that the lytic agent is non-specific and may attack several species of bacteria, although some digest only the bacteria causing the disease and especially when isolated from severe infections.

Properties of the Bacteriolytic Agent.—Considerable emphasis has been placed upon the influence of heat and other physical agents upon the bacteriolytic agent as indicating its nature; that is, whether it is a living organism of ultramicroscopic size or an enzyme.

Twort found that the lytic action of a filtrate was destroyed when heated to 60° C., but not when heated to 52° C. d'Herelle found that it was still active after one hour at 64° to 65° C. Kabeshima² found that a filtrate may be kept at 37° C. for four years, or at less than 0° C. for one hour, or be heated to 70° C. without losing its bacteriolytic power. He found that it was inactivated at 75° C. Davison found that filtrates were unaffected by being heated to 62° to 67° C. for one hour. Kuttner found that her filtrates would withstand being heated to 70° C. for thirty minutes, but that they were inactivated when exposed to 75° C. for thirty minutes. The temperature at which a filtrate and a culture are incubated affects the rate of lysis. d'Herelle noted that it proceeded very slowly at 15° to 16° C. Kuttner found that lysis occurred in about half the time when the filtrate and culture were incubated at 41° to 42° C. as at 37° C. Lysis did not occur when the incubation temperature was 45° to 50° C.

Gratia showed that the inhibition by the lytic agent on the growth of *Bacillus coli* was greatly influenced by the reaction of the medium, being faint at P_H 6.8, 7.0, and 7.4, but much more pronounced at P_H 8.0 and 8.5. Eliava and Pozerski³ stated that filtrates were active in media with reaction from P_H 2.5 to 8.4, but lost their activity when the hydrogen ion concentration was above P_H 2.5 and below 8.4. Davison found that lysis was more complete when the reaction of the medium was at P_H 8.0 and 8.2 than at P_H 6.0 to 7.7. Wollstein reported that the lytic action proceeded as rapidly and as completely in the absence of oxygen as in its presence.

Kabeshima succeeded in chemically precipitating a substance having bacteriolytic power from a stool filtrate. His procedure was to add 3 volumes of acetone to a filtrate, shake, and allow to stand for forty-eight hours at 37° C., and then to decant to remove the acetone. The precipitate was a yellowish powder and had a more powerful bacteriolytic action than the original filtrate. It could be preserved unchanged for six weeks in pure acetone, but its strength diminished after ten weeks. It could be restored, how-

¹ Jour. Bacteriology, 1922, 7, 475, 491.

² Compt. rend. Soc. de biol., 1920, 83, 219, 471.

³ Compt. rend. Soc. de biol., 1921, 84, 708.

ever, by passage through broth cultures of dysentery bacilli. Alcohol also precipitated this bacteriolytic substance. The lytic agent was soluble in ether. By adding an equal volume of anhydrous ether to a filtrate, shaking, allowing to stand forty-eight hours, and then evaporating the ether, a waxy deposit having bacteriolytic activity was obtained. He also found that the addition of 1.0 per cent. sodium fluorid would not destroy the lytic activity of a filtrate, although it has been commonly believed that this amount of fluorid destroyed life and the fermentation associated with life. Bablet,¹ however, found that sodium fluorid inhibited lytic activity and that chloroform and glycerin prevented lytic activity, although they would not prevent the growth of Shiga bacilli. Eliava and Pozerski² stated that twenty-four hours' contact with 2.5 per cent. phenol or 2.5 per cent. fluorid did not affect the bacteriolytic agent, but that 0.75 per cent. quinin chlorhydrate reduced the lytic activity of a filtrate and 1 per cent. quinin chlorhydrate destroyed it. They also claim that quinin salts do not affect soluble ferments.

Maisin³ stated that the lytic substance was completely precipitated by saturating a bacteriolytic filtrate with ammonium sulphate and was almost completely precipitated by half saturation. The fact that the lytic substance would not pass through a collodium membrane led him to assume that it was a colloid. Wollman⁴ has, however, shown that if the membranes are made of dilute collodion (less than 4 per cent.) they are permeable to the bacteriolytic agent. Davison found that lytic activity is destroyed by the addition of 1 c.c. of N sodium hydrate to 4 c.c. of bacteriolytic filtrate.

These properties indicate that the bacteriolytic agent may be an enzyme, but if so, the enzyme requires activation by living bacteria because several investigations have shown that filtrates added to dead bacteria did not produce lysis, although living organisms were quickly dissolved. Apparently only *living organisms are attacked and especially cultures less than twenty-four hours old* in a suitable broth medium rather than suspensions in saline solution. If the bacteriolytic agent is a living organism of ultramicroscopic size it has not yet been successfully cultivated, although it may be active in cultures of dysentery bacilli or other bacteria for many months.

d'Herelle, Davison, and others have found that filtrates containing the bacteriolytic agent are non-pathogenic for rabbits. Antibodies may be produced, however, against the bacterium employed in the preparation of the filtrate due to immunization by the dissolved proteins of the bacteria in the filtrate; antibody may also be produced against the bacteriolytic agent inasmuch as the sera are sometimes antilytic. These results are to be expected, however, and do not yield any particular information regarding the nature of the lytic agent since an antilysin may be produced whether the lysis is a living microbe or a simple enzyme.

Nature of the Bacteriolytic Agent (Bacteriophage; Bacteriolysant).—The exact nature of the bacteriolytic agent is unknown. Twort originally favored the view that it was an autolytic enzyme produced by bacteria and thought that it may arise spontaneously in cultures; more recently he has stated⁵ that d'Herelle's explanation was the more probable.

d'Herelle regards the lytic agent as a diastatic ferment produced in living

¹ Compt. rend. Soc. de biol., 1920, 83, 1322.

² Compt. rend. Soc. de biol., 1921, 85, 139.

³ Compt. rend. Soc. de biol., 1921, 84, 467.

⁴ Compt. rend. Soc. de biol., 1920, 83, 1478; *ibid.*, 1921, 84, 3.

⁵ Brit. Jour. Exper. Path., 1920, 1, 237.

bacteria by the entrance of an organism of ultramicroscopic size. He has called this organism *bacteriophagum intestinale* or *bacteriophage*, the name simply denoting its characteristic property and the place where he first found it. He has summarized his views as follows: "The bacteriophage is an ultramicroscopic organism, which is very widely disseminated in nature. It only grows in contact with living bacteria. It penetrates into the interior of an organism and forms a colony of 15 to 25 elements in the space of one and a half hours. The organism then bursts, liberating the young ultramicrobes. These utilize for their development the bacteria which they dissolve with the aid of the lytic diastase, which they secrete. There is only one species of bacteriophage and this can acquire activity against any organism."

According to d'Herelle the bacteriophage is of an extremely small size capable of passing dense filters, although it may be partially concentrated by prolonged centrifuging at high speed. He has found that it possesses very great vitality, persisting in feces and filtrates properly sealed for several years and in a dried state for as long as six months. It is destroyed by heating at 75° C.; is sensitive to some antiseptics and resistant to others; for example, it has survived in 1 : 200 mercuric chlorid for four days and in phenol 1 : 100 for seven days, but is killed in 90 per cent. alcohol in two days and is highly susceptible to the products of bacteriolysis.

When freshly isolated the bacteriophage usually is capable of attacking several bacterial species, but displays a variable virulence for these. Bacteriophages have been described for the different strains of dysentery bacilli; also for typhoid, paratyphoid, colon, cholera, diphtheria, bubonic plague, and proteus bacilli, and various other bacilli of infectious diseases of the lower animals, as well as *B. subtilis* and staphylococci.

In mixtures of bacterial cultures and bacteriophage some organisms escape lysis and may acquire a resistance to bacteriophage accompanied by morphologic changes. d'Herelle believes that this occurs in the development of "carriers" of dysentery and typhoid bacilli. Some bacteria possess high natural resistance to bacteriophage and especially those micro-organisms possessing high virulence for man or the lower animals and resistant to phagocytosis. According to d'Herelle: "Infection and death or immunity and recovery depend upon whether the bacteria or the bacteriophage triumphs in this battle. The products of the bacteria which have been dissolved by the bacteriophage also play an active rôle in stimulating the formation of antibodies. The outcome of an epidemic also depends upon these two forces for the active bacteriophage, the agent of immunity, as well as the bacteria causing the epidemic can spread from an individual to another."

The lysin or diastatic ferment which d'Herelle claims is produced when the bacteriophage penetrates a living bacterium is precipitable by alcohol, resists heating to 58° C., and is regarded as possessing a powerful opsonic action on the bacteria for which the bacteriophage possesses virulence. d'Herelle claims to have produced antisera for the bacteriophage by injecting filtrates into the lower animals; these antibacteriophagous sera were found to possess complement-fixing antibodies for bacteriophagous antigen and capable of neutralizing but not killing the bacteriophage.

Kabeshima¹ has suggested that the bacteriolysis is due to the interaction of a catalyst present in the feces of the patient and a proferment produced by the bacterium which is attacked and digested. He states that the catalyst is produced by some intestinal gland or by leukocytes as a protective measure against infection and found that the lytic substance could withstand being heated to 70° C. and the effects of precipitating and antiseptic

¹ Compt. rend. Soc. de biol., 1920, 83, 219, 471.

substances, all of which suggests that it is a ferment. d'Herelle has refused to accept this hypothesis not only because in his opinion a living microbe may possess these physical properties, but because it fails to explain serial action by the fact that the same lytic agent can act on diverse bacterial species, etc.

Bordet and Ciuca have suggested that the ability to produce the lytic substance was acquired by bacteria as a result of contact with some external stimulus such as the leukocytic exudate of the peritoneum of a guinea-pig. This external influence possibly represented a defense mechanism on the part of the animal. The bacteria were then able to transmit to their descendants the aptitude to form this lytic substance or this aptitude for autolysis. Inasmuch as this substance was diffusible in a culture-medium, the mere contact with a medium in which such a microbe had grown would confer the ability to produce this substance upon allied bacteria placed on the medium, and these, in turn, to others.

d'Herelle also refuses to accept this hypothesis for various reasons, one of which is that in his opinion it does not conform to the experimental facts.

Bail¹ accepts d'Herelle's claim that the bacteriophage exists in the form of autonomous masses and conducts itself as an ultramicrobe, but states that these particles could only be constituted by the "splitter," that is to say, by particles derived from the digested bacteria themselves. These organized particles, capable of reproduction under a filtrable form at the expense of the same bacteria, secrete a dissolving diastase. d'Herelle states that acceptance of this theory requires strict specificity on the part of the bacteriolytic agent and numerous investigators have proved its non-specificity.

It is apparent, therefore, that the various theories are divided into those regarding the bacteriolytic agent as an enzyme produced by bacteria and those agreeing in principle with d'Herelle, that lysis is indeed caused by a diastatic ferment, but that this ferment is produced by a newly discovered parasite of ultramicroscopic size characterized by its ability to penetrate only living bacteria.

Davison² leans toward the former hypothesis and summarizes the literature and the results of his own experiments as follows: "According to the data available at present d'Herelle's phenomenon probably depends upon a bacteriolytic enzyme produced by bacteria. The amount of this enzyme produced by a culture can be increased by external influences, such as intestinal secretions, tissue extracts, leukocytes, etc. The action of these external influences is probably to favor the development of lysogenic organisms at the expense of the non-lysogenic. This enzyme not only dissolves organisms but also favors the multiplication of bacteria which produce this enzyme. In this way the bacteriolytic principle is carried from generation to generation. It is highly improbable that this phenomenon represents a defense mechanism on the part of an animal against bacterial invasion."

Relation of the Twort-d'Herelle Phenomenon to Immunity.—d'Herelle has placed great importance upon the bacteriolytic agent in relation to infection and immunity. To him the question of whether or not a bacterial disease develops resolves itself into the simple terms of a struggle between infecting microparasite and resisting bacteriophage. The "vicissitudes in the struggle between these two factors are reflected in the condition of the infected individual. Convalescence begins at the moment when the virulence of the bacteriophage is sufficient to give it, definitely, the upper hand.

¹ Wien. klin. Wchn., 1921, 34, 237.

² Abst. of Bacteriology, 1922, 6, 159.

The disease has a fatal outcome if the bacteriophage is inactive as a result of unfavorable conditions, or if the bacterium is able to acquire a refractory state." Since the bacteriolytic agent is regarded as transmissible from one individual to another, d'Herelle believes that it is possible to confer resistance, a heterologous antimicrobial immunity, by administering filtrates containing the agent. He and others have found that these filtrates are well borne by animals in large doses by oral or subcutaneous injection, and from experiments with avian typhoid, barbone, and dysentery d'Herelle states that the administration of the bacteriolytic agent ought to prove curative when administered early in the treatment of disease. As previously stated, Davison, however, was unable to detect any curative activity in dysentery of children; d'Herelle's claims are as yet unproved and the true relation of the bacteriolytic agent to infection, immunity, and the treatment of disease are still as obscure as the nature of the agent itself.

CHAPTER XV

BACTERIAL AGGLUTININS

As previously stated, given any infection, several antibodies of different properties may be produced. If the infecting micro-organism produces characteristically an exogenous toxin, as, for example, that produced by the diphtheria bacillus, an antitoxin is produced as the most prominent of several antibodies. With other pathogenic bacteria that produce mainly an endogenous toxin various antibodies are formed, and one or more may play a prominent rôle in protecting the host, such as opsonins, agglutinins, precipitins, bacteriolysins, etc.

If typhoid immune serum from an immunized animal or a patient suffering from typhoid fever is added to an emulsion of typhoid bacilli in a test-tube and the mixture placed in an incubator, the following phenomenon will be observed: the bacteria, which previously formed a uniform emulsion, clump together into little masses, settle at the sides of the test-tube, and gradually fall to the bottom, the fluid becoming almost clear. In a control test to which no active serum is added, the fluid remains uniformly cloudy. If the reaction is observed microscopically in a hanging drop, it is noted that with the addition of the serum the bacilli move nearer and nearer one another, this process being followed by a gradual loss in motility and the formation of clumps. The substance in the serum causing this phenomenon is called *agglutinin*, and the reaction is known as *agglutination*.

Definition.—*Agglutinins are antibodies that possess the power of causing bacteria, red blood-corpuscles, and some protozoa (trypanosomes) suspended in a fluid to adhere and form clumps.*

Historic.—Although Metchnikoff, Charrin and Roger¹ had noticed peculiarities in the growth of *Bacillus pyocyaneus* when cultivated in immune serum which we now believe were due to agglutinins, Gruber and Durham,² and Bordet³ were the first to recognize that the agglutination reaction was a separate function of immune serum. While investigating the Pfeiffer phenomenon of bacteriolysis with *B. coli* and the cholera vibrio, these investigators found that if the respective immune serums were added to bouillon cultures of these two species the cultures would lose their turbidity, flake-like clumps would form and sink to the bottom of the tube, and the supernatant fluid would become clear. Gruber at the same time called attention to the fact that agglutinins were not absolutely specific for their own antigen, but would agglutinate to a lesser extent closely allied species of bacteria.

In 1896 Pfaundler⁴ drew attention to a peculiar phenomenon observed when bacteria were grown in an immune serum. Long and more or less interlaced threads of bacteria developed, which were regarded as due to agglutinins. At that time considerable emphasis was laid upon the importance of *Pfaundler's reaction*, but at present the ordinary agglutination tests have superseded this reaction as a practical diagnostic procedure.

¹ Compt. rend. Soc. de Biol., 1889, 667; Ann. d. l'Inst. Pasteur, 1891, 5, 473.

² Münch. med. Wchn., 1896, 285; Jour. Path. and Bacteriol., 1897, 4, 13; *ibid.*, 1901, 7, 240; Brit. Med. Jour., 1898, 2, 588.

³ Ann. de l'Inst. Pasteur, 1895, 9, 462.

⁴ Centralbl. f. Bakteriöl., Abt., 1898, 23, 71 and 131.

In 1896 Widal¹ and Grünbaum first turned these facts to practical use in the diagnosis of typhoid fever. These investigators found that the serum of patients suffering from typhoid fever acquires a high agglutinating power for *Bacillus typhosus*, and since this phenomenon generally manifests itself comparatively early in the disease, its recognition has considerable diagnostic importance. It is purely accidental that we speak of the "Widal reaction" in typhoid fever, rather than of the "Grünbaum reaction," for the latter observer conducted similar studies independent of Widal, but, owing to a lack of patients, Widal preceded him in the publication of a more extensive work.

At the present time this diagnostic reaction is known as the *Gruber-Widal reaction*. It has proved of great value to a large number of different investigators, not only in making the serum diagnosis of typhoid fever, but in other infections as well.

Kinds of Agglutinins.—Following the discovery of *bacterial agglutinins* by Charrin and Roger, Bordet² discovered agglutinins in sera for red blood-corpuscles called *hemagglutinins*. Subsequent investigations showed that various substances as phytotoxins (abrin, ricin) and zoötoxins (venoms) possessed the property of agglutinating red corpuscles and particularly those of certain animals called phytagglutins and zoöagglutinins.

In addition to these agglutinins for bacteria and red blood-corpuscles, *myco-agglutinins* may be found in the blood for the higher plants or fungi during mycotic infections, as for *Sporotrichum beurmanni* during sporotrichosis; also protozoa agglutinins for such protozoa as trypanosomes and spirochetes.

Of interest in this connection is the possibility of bacterial agglutinins for some motile bacilli being of two varieties, one for the bodies of bacteria, and the other for flagella. Smith and Reagh,³ working with the hog-cholera bacillus, found body and flagella agglutinins, the latter being much more easily demonstrated in immune sera and manifest in dilutions over twenty times greater than in those in which body agglutinins became visible. These observations were confirmed by Beyer and Reagh,⁴ who also claimed that the flagellar and body (somatic) agglutinins and agglutinable substances of the hog-cholera bacillus may be differentiated by heat.

Hemagglutinins may be present in the blood-serum for the red blood-corpuscles of a different species (*heterologous hemagglutinins*), or for corpuscles of animals of the same species (*homologous hemagglutinins*). The latter are also known as *isohemagglutinins*, as the agglutinin in the serum of one person for the corpuscles of another person. *Autohemagglutinins* occur in the serum for the corpuscles of the same person or lower animal and are rarely found.

Normal and Immune Agglutinins.—Normal serums are frequently capable of agglutinating bacteria, such as the typhoid, colon, pyocyanus, and dysentery bacilli. In some cases the typhoid bacillus may be agglutinated in dilutions as high as 1 : 30, a point of practical importance in the clinical use of the test. When a normal serum is found to have a high agglutinating power, it is probable that a previous infection by the micro-organism has occurred. Since the serum of a newborn child is largely devoid of agglutinins that are found in later life as shown by Savage⁵ and others, the so-called *normal* or *natural agglutinins* may, after all, be acquired properties.

¹ Semaine méd., 1896, 259.

² Jour. Med. Research, 1903, 1904, 10, 89.

² Ann. d. l'Inst. Pasteur, 1898, 12, 688.

⁴ Jour. Med. Research, 1904, 12, 313.

⁵ Jour. Hyg., 1918, 17, 34.

The term *immune agglutinin* is applied to the agglutinating substance in a serum developed as the result of infection or of systematic immunization with the micro-organism.

Studies in the bacterial agglutinins have been almost entirely confined to the sera of warm-blooded animals; recently, Takenouchi¹ has shown that the sera of several varieties of turtles may contain agglutinins for various bacteria of the typhoid-colon group. These observations are also of interest from the standpoint of origin of normal agglutinins, as their formation for these bacilli in cold-blooded animals by unrecognized infection appears improbable.

As shown by Noguchi² the sera of many cold-blooded animals may contain normal agglutinins and hemolysins for the corpuscles of cold-blooded animals; since then Friedberger and Seeling,³ Landsteiner and Rock,⁴ Fränkel,⁵ Mazzetti,⁶ Takenouchi,⁷ and others have demonstrated hemagglutinins and

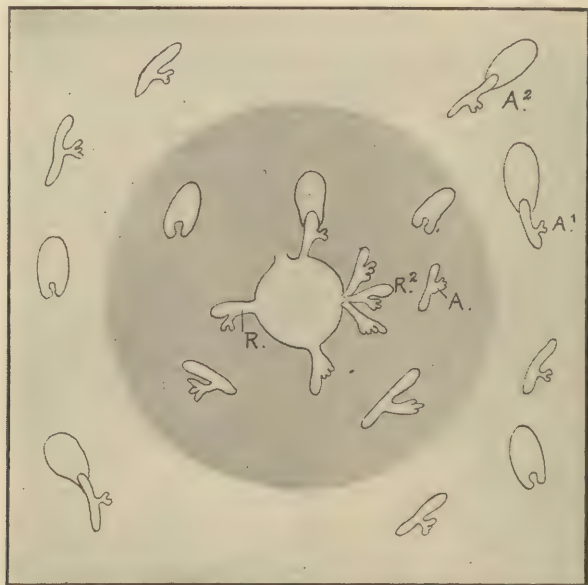


FIG. 95.—THEORETIC FORMATION OF AGGLUTININS.

hemolysins in the sera of cold-blooded animals for the corpuscles of various cold- and warm-blooded animals.

Agglutinins and Agglutinoids.—According to Ehrlich's side-chain theory, agglutinins are antibodies of the second order (Fig. 95). They resemble antitoxins or receptors of the first order in possessing an affinity-bearing or haptophore group that unites with the antigen, but they differ from them in having also a functional or agglutinophore group that agglutinates the antigen when this union has occurred (Fig. 96).

Agglutinins that have lost their zymophore or agglutinophore group through the action of heat, age, acids, etc., but that still possess their haptophore group, are called *agglutinoids*, just as toxins that have lost their toxo-

¹ Jour. Infect. Dis., 1918, 23, 393.

² Bull. Univ. of Penna., 1902, 14, 438.

³ Centralbl. f. Bakteriöl., orig., 1908, 46, 421.

⁴ Ztschr. f. Immunitätsf., 1912, 14, 14.

⁵ Ztschr. f. Immunitätsf., 1911, 10, 415.

⁶ Ztschr. f. Immunitätsf., 1913, 18, 132.

⁷ Jour. Infect. Dis., 1918, 23, 415.

phore group are called toxoids. Such agglutinoids, then, may still combine with the bacteria or blood-cells without being able, however, to produce agglutination (Fig. 97). Heating for thirty minutes at 65° to 80° C. usually changes agglutinins to agglutinoids; agglutinoids are destroyed at temperatures above 80° C.

It is found, at times, that even a fresh serum when concentrated will cause less agglutination than when it is diluted. This is ascribed to the presence of agglutinoids, which have a stronger affinity for agglutinin than has the agglutinin. When producing a reaction of this character they are called *proagglutinoids*. When the serum is diluted, the proagglutinoids become less concentrated and finally, when they are diluted as to have no influence on the reaction, the agglutinins are still present in sufficient quantity to bring about agglutination. As a practical fact, in agglutination reactions the action of proagglutinoids is of much importance, for the inexperienced may be misled by the absence of, or by poor, agglutination in lower dilutions to neglect the use of higher dilutions.

Agglutinin.—The substance in bacteria or other cells that produces agglutinin is called *agglutinin*. It appears to be formed in the cell, and in some cases may be excreted into the surrounding medium. Certainly

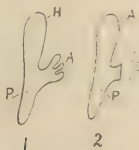


FIG. 96.—THEORETIC STRUCTURE OF AGGLUTININ AND AGGLUTINOID.

1, Agglutinin: *H*, Haptophore group for union with antigen; *A*, the agglutinophore or zymophore group.

2, Agglutinoid. Same structure as agglutinin, except that the agglutinophore or zymophore group is lost.

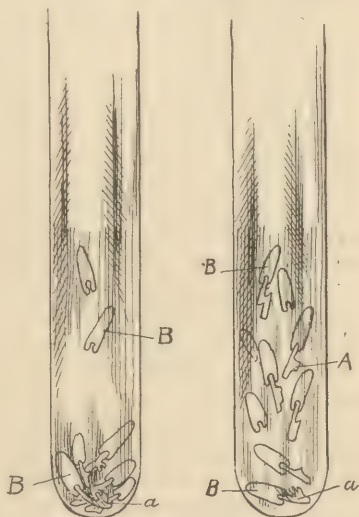


FIG. 97.—A DIAGRAMMATIC ILLUSTRATION OF THE ACTION OF AGGLUTININS AND AGGLUTINIDS.

In the first tube (left) most of the bacilli (*B*) have been agglutinated and massed in the bottom of the tube by the agglutinins (*a*).

In the second tube (right) the bacilli (*B*) are in combination with the agglutinoids (*A*), but agglutination does not occur because the agglutinophore groups are lost. A few bacilli have been agglutinated by the agglutinins (*a*).

when bacteria die and become disintegrated, agglutinin is liberated and the filtrates (entirely free from bacterial cells), when injected into animals, will cause the formation of agglutinins. The term *agglutinin* is also used for designating the antigen or suspensions of cells used in conducting agglutination tests.

Agglutinin must be considered as having a simple haptophorous group, through which it may unite with the receptors of the tissue cells. This haptophore comes into play again in the union between agglutinin and agglutinin, which precedes agglutination. It is a passive body, similar to the haptophore of antitoxin, and has no other function than that of uniting either with cell or with agglutinin.

Origin and Distribution of Agglutinins.—The investigations that have been carried out for the purpose of determining the site of formation of

agglutinins have not thus far yielded conclusive results. The lymphoid tissues appear especially concerned, agglutinins being found early in the bone-marrow and the spleen by Pfeiffer and Marx¹ and van Emden.² Metchnikoff believes that agglutinins may be derived from leukocytes and endothelial cells. It is more probable, however, that the formation is general, and is the result of wide-spread cellular activity.

In accordance with the side-chain theory the ability of an animal to form agglutinins for a certain micro-organism would depend on its possession of receptors of the second order, which are able to unite with the agglutinogenic receptors of the micro-organism. It has been well established that the number of such suitable receptors vary in animals, and that different animals may not produce serums with equal agglutinating powers.

Agglutinins do not appear in the serum immediately after inoculation, but require an incubation period of from two to four days for their production.

Agglutinins are to be found in highest concentration in the blood. Dreyer and Walker³ found that the plasma contained slightly more normal or natural agglutinins than the corresponding serum; during immunization, however, more agglutinins were found in the serum. Cerebrospinal fluid is free of normal or natural agglutinins; during typhoid fever, and other infections accompanied by great production of immune agglutinins, small amounts of the antibody may be found in this fluid. Blister fluid, exudates, transudates, milk, and tears may contain agglutinins if these are present in the blood, as shown by Widal⁴ in typhoid fever. As recently shown by Little and Orcutt,⁵ the agglutinins toward *Bacillus abortus* found in the blood-serum of newborn calves are obtained from the mother through the colostrum.

Properties and Nature of Agglutinins.—1. Agglutinins are fairly resistant substances that withstand heating to 60° C. for thirty minutes and lose their power only when heated to higher temperatures. It is possible, therefore, to make a serum bacteriolytically inactive by destroying complement at 55° C., and still retain its agglutinating power.

2. They resist drying, and their activity is best preserved in this state. They do not dialyze through animal membranes.

3. As shown by Bechhold,⁶ Field and Teague,⁷ and others agglutinins are electro-positive, that is, bacteria move toward the anode under the influence of an electric current. Field and Teague have also shown that a combination of bacteria and agglutinin may be partly disassociated by means of the electric current.

4. They are precipitated from a serum by magnesium or ammonium sulphates, when these salts are used in proper concentration, and are thus closely associated with the globulin fraction of serum.

5. They are separate and distinct antibodies, and are not associated with bacteriolysins. Thus, the agglutinins of an immune serum may be lost, destroyed, or absorbed and the bacteriolysins retained. As previously mentioned the bacteriolytic power of a serum may be inhibited by heating it to 55° C. for a half hour without influencing the agglutinin content, and during disease processes the formation of agglutinins and that of bacteriolysins are apparently not parallel processes.

¹ Ztschr. f. Hyg., 1898, 27, 272.

² Ztschr. f. Hyg., 1899, 30, 19.

³ Jour. Path. and Bacteriol., 1910, 14, 39.

⁴ Semaine Med., 1896, 259.

⁵ Jour. Exper. Med., 1922, 35, 161.

⁶ Ztschr. f. physik. Chem., 1904, xlviii, 385.

⁷ Jour. Exper. Med., 1907, 9, 222.

Stuber¹ has claimed that agglutinins are lipoidal and may be extracted from sera with petroleum ether; Krumwiede and Noble,² however, were not able to confirm these observations and found no evidence supporting the claim that agglutinins are lipoidal in character. In this connection it may be stated that Graham³ has shown that ether anesthesia has no influence upon agglutinins.

Acid Agglutination.—Bacteria may be agglutinated by acids, and the method of acid agglutination was introduced by Michaelis⁴ for the differentiation of bacterial species on the basis that the hydrogen-ion concentration at which agglutination is maximal is characteristic for various species of closely allied types. The results of considerable investigation on the acid agglutination of the typhoid-colon group of bacilli has shown that *Bacillus typhosus* and *B. paratyphosus* are readily distinguished by means of the reaction. Michaelis⁵ believes that his acetic-acid method has proved superior to serum-agglutination reactions for the differentiation of the typhoid-paratyphoid-dysentery coli group of bacilli. Definite differentiation between the paratyphoid bacilli A, B, and C have not been seen by Beniasch,⁶ Jaffe,⁷ Heinmann,⁸ and Grote⁹; Beniasch has also reported the resistance of *B. coli* to acid agglutination at any hydrogen-ion concentration and, indeed, he has found certain strains of nearly all species of bacteria to be non-agglutinable within the tested reaction limits. Gillespie¹⁰ found that pneumococci belonging to the serologic types I and II have, as a rule, narrow zones of agglutination. The optimum hydrogen-ion concentration was found different in the two cases, while other pneumococci had broad zones or, in a few cases, narrow zones not coincident with those occupied by the typical organisms. For a technic of acid agglutination see page 289.

Mechanism of Agglutination.—The true nature of the phenomenon of agglutination is unknown, as is shown by the number of theories advanced. Thus:

1. Gruber's¹¹ idea of the mechanism of this phenomenon was that the agglutinin so changed the bacterial membrane as to render it more viscous, and that this increased viscosity caused the bacteria to adhere and form clumps. No visible changes in the organisms or red corpuscles can, however, be seen.

According to the investigations of Malvoz,¹² Dineur,¹³ Nicolle and Treuel,¹⁴ the flagella of bacteria are intimately concerned in the phenomenon of agglutination and that the bacilli become attached to one another by means of these cilia. Ernst and Roby,¹⁵ however, have not been able to confirm these findings and do not regard the flagella of bacteria as being vitally concerned in the phenomenon. Furthermore, it is well known that non-motile and flagella-free bacteria may be agglutinated, but the subject is

¹ Münch. med. Wchn., 1915, 62, 1173; Biochem. Ztschr., 1916, 77, 273.

² Jour. Immunology, 1921, 6, 201.

³ Jour. Infect. Dis., 1911, 8, 147.

⁴ Deutsch. med. Wchn., 1911, 37, 969.

⁵ Deutsch. med. Wchn., 1914, 41, 241.

⁶ Ztschr. f. Immunitätsf., orig., 1912, 12, 268.

⁷ Arch. f. Hyg., 1912, 76, 1.

⁸ Ztschr. f. Immunitätsf., orig., 1913, 16, 127.

⁹ Centralbl. f. Bakteriöl., etc., orig., 1913, 69, 98.

¹⁰ Jour. Exper. Med., 1914, 19, 28.

¹¹ Wien. klin. Wchn., 1896, 183, 204.

¹² Ann. de l'Inst. Pasteur, 1897, No. 6.

¹³ Bull. d. l'Acad. Roy. d. Med. d. Belgique, 1898, 12, 705.

¹⁴ Ann. d. l'Inst. Pasteur, 1902, 16, 562.

¹⁵ Trans. Cong. Amer. Phys. and Surg., 1900, 26.

of interest in view of the investigations of Smith and Reagh,¹ who found that agglutinin acting upon the bodies of hog-cholera bacilli may be different from those acting upon the flagella.

2. Paltauf's theory is somewhat similar, he believing that the agglutino-gen is precipitated on the surface of the bacteria by union with the agglutinin, with the formation of a sticky substance. He cites evidence that tends to show that such substances are actually thrown out from the bacteria during agglutination, as may be seen in a properly stained preparation in the form of a precipitate surrounding the bacterial cells.

3. The presence of some salt is necessary for the occurrence of agglutination. Bordet² found that if the salts were removed from the serum and from the suspension of bacteria by dialysis and that the two were then mixed, agglutination did not occur, but that if a small amount of sodium chlorid was added, agglutination promptly took place. According to this view, therefore, agglutination is a phenomenon of molecular physics—the agglutinin acts on the bacteria or other cells and prepares them for agglutination by altering the relations of molecular attraction between them and the surrounding fluid, the second phase, the loss of motility, clumping, etc., being brought about by the presence of salt. This second phase, therefore, would be a purely physical phenomenon, the salts altering the electric conditions of the colloidal-like agglutinin-bacterium combination, so that their surface tension is increased. To overcome this the particles adhere together, presenting in a clump less surface tension than if they remained as individual particles. Bordet cites the precipitation of clay as an analogous case: if a little salt is added to a fine emulsion of potters' clay in distilled water the clay immediately clumps and falls to the bottom, the resemblance between these flakes and the clump of agglutinated bacteria being very striking. Support for these findings has been furnished by the studies of Crenidropoulos and Amos,³ of Landsteiner,⁴ and Lange.⁵ The physicochemical nature of agglutination is also indicated by the interesting observation of Bond,⁶ who found that the agglutinin content of sera for erythrocytes and bacteria may be altered (generally increased) by mechanical processes embracing friction and pressure of dried sera.

Specificity of Agglutinins.—For a time after their discovery the agglutinins were regarded as strictly specific, *i. e.*, a typhoid-immune serum would agglutinate only typhoid bacilli and no others. Gruber early pointed out that an immune serum will frequently agglutinate other closely related organisms, although not usually to so high a degree.

Group or partial agglutinins, therefore, refer to the presence in a serum of certain agglutinins that agglutinate certain other micro-organisms that are morphologically, biologically, and often pathogenetically closely related to the homologous micro-organism (the bacterium causing the infection or used in artificial immunization). For example, a typhoid-immune serum possesses, besides its greatly increased agglutinating power for *Bacillus typhosus*, some agglutinin for *B. paratyphosus*, notably above that of normal serum. This is explained by the very close biologic relationship of these bacteria, together with the fact that the agglutinin-producing substance (agglutino-gen) is a complex and not a single chemical substance. This has been explained by Durham in the following example: If the typhoid

¹ Jour. Med. Research, 1903, 1904, 10, 89.

² Ann. d. l'Inst. Pasteur, 1899, 13, 225.

³ Jour. Path. and Bacteriol., 1904, 9, 260.

⁴ Ztschr. f. Immunitätsf., orig., 1910, 8, 397.

⁵ Ztschr. f. Immunitätsf., orig., 1915, 24, 587.

⁶ Brit. Med. Jour., June 14, 1919.

agglutinin is composed of various elements, A, B, C, D, it is conceivable that the closely related paratyphoids might contain one or more of these four agglutinogens and, therefore, the agglutinating power of the typhoid serum for a paratyphoid bacillus, though not so great as on the typhoid bacillus, is still considerable. Accordingly, in an infection with one micro-organism a specific agglutinin will be formed for that micro-organism, and group agglutinins for other more or less allied micro-organisms, and consequently the specificity of the agglutinating reaction depends upon the principle of dilution, the specific agglutinin being present in largest amount and operative in dilutions above the range of the group agglutinins.

Absorption Methods for Differentiating Between a Mixed and a Single Infection.—In 1902 Castellani¹ discovered that if the serum of an animal immunized against a certain micro-organism contains that micro-organism in large numbers the serum will lose its agglutinating power, not only for that micro-organism, but also for all other varieties on which it formerly acted. If, however, the serum contains the organism corresponding to the group agglutinins, the agglutinating power of the serum for the homologous organism is reduced but little or not at all.

In a mixed infection, due to two or more varieties of bacteria, there will be specific agglutinins for each of the micro-organisms and group agglutinins for each of them as well. If the immune serum is saturated with one of these varieties its chief or major agglutinins and some or all of the group agglutinins will be removed, but the major agglutinin of the second species will remain. On the addition of the second bacterium to the immune serum agglutination occurs and its agglutinin is absorbed. Park, who has carefully investigated this subject, finds that the absorption method proves that when one variety of bacteria removes all agglutinins for a second, the agglutinins in question were not produced by the second variety.

Non-agglutinable Species of Bacteria.—Certain species of bacteria, especially when freshly isolated from the animal body, may prove themselves immune to the action of agglutinins; this is especially true of the bacillus of Friedländer. The isolation of inagglutinable or feebly agglutinating strains of typhoid bacilli has been recorded by Achard and Bensaude,² Kolle,³ Johnston and Taggart,⁴ Sacquepee,⁵ Klinger,⁶ Lesieur,⁷ Buxton and Vaughan,⁸ and others. It would appear that inagglutinability arises as the result of an increased resistance on the part of the bacilli to antibodies secreted against them; there is no evidence to show that there is any relation between this state and chronicity of infection. As a rule, this resistance is lost when the micro-organism is grown for some time in artificial media. In some instances the typhoid bacillus, when freshly isolated from a patient, may resist agglutination until after it has passed a period of existence on artificial media. This variability is probably due to some change taking place in the agglutinable substance of the agglutinins during the sojourn of the bacilli in the animal body, and possess such an excess of agglutinogenic receptors as to require a much larger amount of agglutinin to cause agglutination.

McIntosh and McQueen⁹ have isolated a strain of inagglutinable typhoid

¹ Ztschr. f. Hyg., 1902, 11, 17.

² Compt. rend. Soc. de biol., 1896, xlv, 940.

³ Deutsch. med. Wchn., 1897, 23, 132.

⁴ Montreal Med. Jour., 1897, 25, 709.

⁵ Ann. d. l'Inst. Pasteur, 1901, 15, 249.

⁶ Centralbl. f. Bakteriöl., orig., 1902, 32, 542.

⁷ Jour. d. physiol. et de path. gén., 1903, 5, 539.

⁸ Jour. Med. Res., 1904, 12, 115.

⁹ Jour. of Hyg., 1914, 13, 409.

bacilli from a case of typhoid fever and made experiments with it, the results of which throw light on this question of inagglutinability. They found, and others have made similar observations, that the inagglutinable strain, when injected into animals, led to the production of typhoid agglutinins which had practically no action on itself but agglutinated heterologous typhoid strains freely; and, furthermore, that the strain in question would absorb typhoid agglutinins just like the agglutinable strains. Hence, the inagglutinability cannot be due to loss of affinity for the agglutinins, that is, loss of receptors in the terms of the side-chain hypothesis, but rather to some physical alteration by virtue of which the second step in agglutination fails to take place, namely, aggregation or clumping. They appear to have no peculiarities with respect to complement fixation, and are clumped by chemical agglutinants, especially acids, an observation showing that acid and serum agglutination do not depend on the same factors.

It should be remembered that agglutinins act on dead as well as on living bacteria, those killed by heat, formalin, phenol, etc., being similarly agglutinable. In making the microscopic test the use of dead bacteria is not so satisfactory as when the test is made with living motile bacteria, for the influence of the serum on motility alone is of value in interpreting a reaction.

Variation in Agglutinating Strength of a Serum.—In a given infection, such as typhoid fever, there is usually a continued increase in the amount of agglutinin in the blood from the fourth day until convalescence is established, and then a decrease occurs. It is a fact of practical importance that the agglutinating power of a serum may vary from day to day, so that it is very strong one day, and may become weak or disappear entirely on the next day or two. Hence, the importance of making more than one test in a suspicious case when the first trial has been doubtful or negative. There is no satisfactory explanation for this variation, mixed infection, intestinal hemorrhage, etc., being regarded by some as responsible for it.

Conglutination.—In 1906 Bordet and Streng,¹ and Bordet and Gay² described a colloidal substance in beef-serum heated to 56° C. ("bovine colloid") which has the property of causing a characteristic clumping and increased lysis of red blood-cells when treated with a heated specific hemolytic serum and fresh alexin (complement). Bordet and Streng, in later studies on this substance, gave to it the name "conglutinin." Streng³ continued these studies with bacteria and found that a typical clumping was produced by the mixture of bacteria, fresh complement, conglutinin, and a specific immune serum from which the agglutinins had been removed by absorption. By dialyzing the beef serum the conglutinin was shown to be present in the globulin fraction, and the reaction took place as well with bacteria killed by heat or 0.1 per cent. liquor formaldehydi as with live organisms.

In a study of dysentery in infants Lucas, Fitzgerald, and Schorer⁴ first applied this reaction to clinical diagnosis. They found it more sensitive and specific than either the agglutination or fixation test.

In their work cultures of the Flexner and Shiga dysentery bacilli treated with 0.1 per cent. liquor formaldehydi were used. They conclude that in the conglutination test we have a means of diagnosis far superior to any other form.

¹ Centralbl. f. Bakteriöl., 1909, xlix, 260.

² Ann. d. l'Inst. Pasteur, 1906, xx, 467.

³ Centralbl. f. Bakteriöl., 1909, l, 47; *ibid.*, 1909, 2, 415.

⁴ Jour. Amer. Med. Assoc., 1910, lvi, 441.

Swift and Thro¹ did not find the conglutination reaction of much greater value in the differentiation of various strains of streptococci than the agglutination reaction.

Karvonen attempted to modify the Wassermann test for syphilis by adopting the principles of conglutination instead of hemolysis, but numerous investigations by Seibert and Mironescu,² Hect,³ Leschly and Boas,⁴ and others have shown the method to be unsatisfactory. Maltauer and Johnston⁵ have recently shown that the reaction is due to fibrinogen and a heat-sensitive serum constituent; further reference to conglutination will be made in the chapter on Cytolysins.

The technic of this reaction is given on page 285. Leschly⁶ describes technical details and gives a complete review of literature.

Rôle of Bacterial Agglutinins in Immunity.—The agglutinins were formerly regarded as possessing a true protective and curative power by Max Gruber and others. However, Gengou⁷ was unable to establish any relation between the agglutinative power and the refractory state of animals to anthrax. In his experiments it was found that human serum may contain large amounts of agglutinin for attenuated anthrax bacilli and yet man is far from being immune to anthrax. Pigeon's serum, on the other hand, is free of agglutinins for anthrax bacilli and yet this animal enjoys a high degree of natural immunity.

It has previously been mentioned that bacteria may be grown in a specific agglutinating serum, and cultures made of agglutinated bacteria show them to be fully alive and as virulent as before agglutination took place. In certain cases agglutinins for a micro-organism may be entirely absent and yet the animal enjoy an immunity. Bacteria that have been acted upon by an agglutinin are apparently not altered in appearance, viability, or virulence, as shown by the experiments of Issaeff⁸ in Metchnikoff's laboratory with the pneumococcus, and by Sanarelli,⁹ and Mesnil¹⁰ with vibrios and the bacillus of swine erysipelas.

Many observations tend to show that the agglutinating power of a serum gives no indication of the degree of immunity that exists. For instance, relapses may occur in typhoid fever at a time when the agglutinating power of the patient's blood is at its highest, as first shown by Widal and Sicard.¹¹

At present agglutinins are regarded as playing a subsidiary rôle in immunity, their presence being of diagnostic value, and an indication of the presence of more important factors, and as an aid to bacteriolysis and phagocytosis, as first shown by Besredka¹² with guinea-pigs infected with typhoid bacilli.

As shown by Bull¹³ experimentally agglutination may occur *in vivo*, and the power of the blood to cause agglutination determines, in large measure, whether, after their direct introduction in an experimental way, the bacteria are to be removed promptly from the circulation and bacteremia avoided,

¹ Archiv. Int. Med., 1911, 7, 24.

² Deutsch. med. Wchn., 1911, 37.

³ Berl. klin. Wchn., 1912, 49, No. 2.

⁴ Hospitalst., 1913, 57, 1201.

⁵ Jour. Immunology, 1921, 6, 349.

⁶ Ztschr. f. Immunitätsf., orig., 1916, 25, 219.

⁷ Archiv. Intern. d. Pharmacodyn., 1899, 6, 299; Ann. d. l'Inst. Pasteur, 1899, 13, 642.

⁸ Ann. d. l'Inst. Pasteur, 1893, 7, 260.

⁹ Ann. d. l'Inst. Pasteur, 1893, 7, 225.

¹⁰ Ann. d. l'Inst. Pasteur, 1898, 12, 481.

¹¹ Ann. d. l'Inst. Pasteur, 1897, 11, 411.

¹² Ann. d. l'Inst. Pasteur, 1901, 15, 209.

¹³ Jour. Exper. Med., 1915, 22, 475, 484; *ibid.*, 1916, 24, 7, 25, 35; *ibid.*, 1916, 23, 419.

or whether they are to remain and produce bacteremia. Micro-organisms which are not agglutinated in the normal rabbit may be made to do so by the intravenous injection of homologous immune serum, and this probably explains the rapid disappearance of pneumococci from the blood following the intravenous injections of homologous antipneumococcus serum. The bacterial clumps accumulate in the organs in which they are phagocyted. In all of Bull's investigations, including instances of both natural and passive immunity, the agglutination of bacteria in the blood of the infected animal was followed by phagocytosis and destruction of the bacteria in the viscera of the body with a subsequent disappearance of the bacteria from the blood-stream. It was observed, however, that when agglutination was incomplete, a few micro-organisms remained in the blood, later to multiply and cause a fatal bacteremia. In this way it appears that agglutinins, opsonins, and phagocytosis are closely related and probably exert an important rôle in resistance to, and recovery from, infection.

PRACTICAL APPLICATIONS

The agglutination reaction is used for the following purposes:

1. For the **diagnosis of disease**, by identifying the bacterial infection from which the patient is suffering. To do this satisfactorily we must have on hand stock cultures of bacteria, and test the patient's serum for agglutinins for these bacteria. For instance, if a patient presents symptoms of typhoid fever, the serum is tested for typhoid agglutinins; if the reaction is very weak or negative and continues so the serum is further tested for agglutinins for *Bacillus paratyphosus* A and B.

2. Agglutination reactions are also of value as an aid to the **identification of a micro-organism** that has been cultivated from a patient. For this purpose we must have on hand various standard immune serums. For example, if a bacillus resembling the typhoid bacillus is isolated from the feces of a patient the diagnosis may be aided by a positive agglutination reaction with typhoid immune serum. Reference has been made to the use of the test for the differentiation of pneumococci. The agglutination test is also of great value in the identification of meningococci, and in the differentiation of the three groups of this micro-organism. Similar studies have been made in the serologic grouping of streptococci and gonococci to which further reference is made in the chapter on Serum Therapy.

3. Agglutination tests are of value in determining whether in a case in which more than one micro-organism has been cultivated the condition at hand is a **single or a mixed infection**. The absorption agglutinin test is made with the patient's serum and the cultures are isolated from the patient.

4. Agglutination tests are also of value for measuring the **immunizing response** that a patient is making to his infection or to artificial immunization. Thus, the test is of some value in determining the response to inoculation with typhoid vaccine, although it is probable that the agglutinin itself does not possess true antimicrobial properties.

Agglutination in Typhoid and Paratyphoid Fevers.—In this group the agglutination test has proved of great value in diagnosis; probably the microscopic method employing serum or dried blood is mostly employed, but recent experiences with the macroscopic technic employing cultures prepared according to the Dreyer method have indicated that the latter is more delicate and more reliable and accurate.

In *typhoid fever* the Gruber-Widal reaction may be positive as early as

the third day; usually, however, the positive reaction is obtained somewhat later—about the seventh or the eighth day. A day or so earlier the bacilli used in making the test may be seen to lose their motility and two or three may form a loose clump. This is the doubtful reaction, and it is well to test every day or every other day until a decisive reaction is obtained.

According to Park, "about 20 per cent. of typhoid infections give positive reactions in the first week; about 60 per cent. in the second week; about 80 per cent. in the third week; about 90 per cent. in the fourth week, and about 75 per cent. in the second month of the disease." In about 90 to 95 per cent. of cases in which repeated examinations are made a positive reaction is to be found at some time during the patient's illness. Moreschi¹ found that in 6.7 per cent. of cases agglutinins may not be found.

Occasionally the reaction appears first during the stage of convalescence, and at times it may even be absent, the diagnosis being confirmed by cultivating typhoid bacilli from the blood. The possibility of a given case reacting strongly one day and weakly or entirely negative a day or so later has been emphasized elsewhere. Dreyer and Walker² found that the agglutinins reached the maximum in typhoid and paratyphoid fever between the sixteenth and twenty-fourth day of the disease.

Usually the reaction is strongest during convalescence, remains positive for several weeks, and then gradually returns to the normal. Occasionally the reaction remains positive for months or even years after the attack of typhoid fever; many such cases are "carriers" and harbor the bacilli in the gall-bladder, although the person appears to be quite well.

Only very rarely does normal serum immediately agglutinate typhoid bacilli in a dilution higher than 1 : 10; where a time limit of one to two hours is given a few may show some agglutination in dilutions up to 1 : 30.

If the typhoid bacillus is agglutinated by the patient's serum in a dilution of 1 : 100, or at least 1 : 30, the Widal reaction may be regarded as positive. It is not safe to use lower dilutions, as occasionally the serum of healthy persons may agglutinate *Bacillus typhosus* in dilutions up to 1 : 25.

Ritchie³ has studied the normal agglutinins in human sera with much care and states:

Complete agglutination in a dilution of 1 : 16 should be looked on with considerable suspicion; complete agglutination in dilution of 1 : 32 or above should be looked on as diagnostic. The same dilutions were given for the paratyphoid bacilli.

Due care must be exercised not to mistake a pseudoreaction about detritus for true agglutination.

Positive reactions are occasionally obtained in other diseases—acute military tuberculosis, malaria, malignant endocarditis, and pneumonia. It is also well to bear in mind the possibility of a patient having been vaccinated against typhoid at some early date with resulting agglutinin formation.

The agglutination test is of particular value as an aid in the diagnosis of the mild typhoid or paratyphoid infections so apt to be mistaken for some other disease and the detection of which is of great importance to the community. Blood culture should be performed at once and agglutination tests at frequent intervals.

Owing to the similarity of symptoms an infection with *Bacillus paratyphosus* A and B may be difficult to distinguish from typhoid fever. This difficulty is increased by the confusion of the Widal reaction owing to the

¹ Ztschr. f. Immunitätsf., orig., 1914, 21, 410.

² Lancet, London, 1916, September 2.

³ Lancet, London, 1916, 1, 1245.

presence of group agglutinins in the serum if proper dilution is not practised. Bacillus A and Bacillus B are not identical in their agglutinable properties, the latter being more closely related to the typhoid bacillus than the former. In this country *B. paratyphosus* is usually held responsible for paratyphoid fever. Conclusions should not be drawn until tests have been made with both strains of the paratyphoid bacillus and with the typhoid bacillus. In conducting the tests for paratyphoid fever strains of both *B. paratyphosus* A and B should be employed; positive reactions with dilutions of 1 : 30 or higher are significant and in most instances diagnostic, providing the patient has not previously received triple typhoid-paratyphoid vaccine. Dreyer¹ believes that an agglutination of 1 in 10 is for all practical purposes diagnostic in cases of paratyphoid A. In case of mixed infection the absorption method of Castellani will serve to clear up the diagnosis.

Agglutination Reactions for the Detection of Typhoid Carriers.—Bigelow² and others have shown that a persistently high agglutinin titer of the serum after typhoid fever indicates a carrier condition; the agglutination reaction may prove of value in the detection of carriers in addition to bacteriologic examination of the bile, feces, and urine. Sequelæ of typhoid fever, as periostitis and cholecystitis, are usually accompanied by a persistently high titer of the serum in agglutinins.

Agglutination Reaction After Typhoid-paratyphoid Immunization.—It is well established that the administration of typhoid or mixed typhoid-paratyphoid vaccine is followed by the production of agglutinins. Even a single dose of vaccine results in agglutinin production; after two or three doses the agglutinating titer of the serum is usually, but not always, further increased.

The production of agglutinins by vaccines is of great importance in relation to the use of the agglutination test for diagnosis purposes; the physician should always inquire into this phase of a patient's history. If vaccines have been received the ordinary Widal test is apt to be worthless in diagnosis; more accurate quantitative agglutination reactions are required as described below.

Vaccinated individuals if quite recently inoculated will usually show a high titer of specific agglutination. A rapid rise in titer sets in within two to four days of inoculation. This is followed by a fall at first rapid, but subsequently becoming very slow so that a relatively high titer is maintained for a long period (even for years). During this period examinations made at intervals of a few days give practically identical readings.

Dreyer and Inman,³ Dakeyne,⁴ and Krumbhaar and Smith⁵ found that the agglutinins after vaccination persist for at least eight to twelve months and frequently for longer periods. However, the rate of disappearance of these agglutinins varies greatly in different persons. As stated by Meyer and Kilgore⁶ on the basis of their own work and a review of the literature up to 1917, it is not possible to make any statement in regard to the rate with which agglutinins disappear from the blood of vaccinated individuals.

It is entirely likely that immunity to typhoid fever persists for some time after the agglutinins in the blood have decreased to normal proportions. It is customarily stated that vaccination protects for two or three years. Not infrequently individuals inquire at the expiration of this time

¹ Proc. Roy. Soc. Med., 1915, Medical Section, 10.

² Jour. Amer. Med. Assoc., 1911, 57, 1418; *ibid.*, 1912, 58, 339.

³ Lancet, London, 1915, 2, 225.

⁴ Lancet, London, 1915, 2, 540.

⁵ Jour. Infect. Dis., 1918, 23, 126.

⁶ Archiv. Int. Med., 1917, 19, 293.

if revaccination should be done; it is my practice to first titrate the agglutinin content of the blood and advise revaccination only in case the titer for typhoid bacilli is 1 : 30 or less, and for the paratyphoid bacilli if the titer is 1 : 20 or less.

Agglutination Reactions in the Diagnosis of Typhoid and Paratyphoid Fevers in Vaccinated Individuals.—The wide-spread use of typhoid-paratyphoid immunization has introduced many new difficulties in the use of the agglutination test for the diagnosis of typhoid and paratyphoid fevers among immunized individuals. Blood, feces, and urine cultures should always be resorted to if possible for diagnosis, but these may show the presence of the bacilli in about slightly more than 50 per cent. of cases.

The ordinary Widal test is almost worthless under these circumstances unless it is definitely known that the agglutinins had dropped to normal levels prior to the onset of enteric symptoms. Under these circumstances a more accurate quantitative method is required, employing a culture of uniform and unchanging properties as the formalized suspensions prepared by the Dreyer method, in order that the results of three or more successive observations shall be strictly comparative.

Tidy¹ has claimed that febrile conditions destroy the agglutinins produced by vaccination, and that "a positive agglutination reaction to *Bacillus typhosus* after the fifth day of pyrexia is a definite proof of typhoid fever in an inoculated man as in a non-inoculated one." Dreyer and Walker,² Donaldson and Clark,³ Wilson,⁴ and others have challenged these statements and have apparently proved that Tidy's comments are erroneous. Indeed, Conradi and Bielings⁵ have stated that just the reverse may occur, and that intercurrent infections, as tuberculosis, may actually increase the typhoid agglutinins to a slight degree and that agglutination tests may lead in this way to an erroneous diagnosis. The results reported by Perry⁶ and other English investigators referred to above have indicated, however, that a definite increase of agglutinins detected by an accurate method is due to enteric infection.

It follows that in the case of inoculated persons the diagnosis of active typhoid (or paratyphoid) infection will require two or more successive examinations of the serum.

- (a) If the individual is suffering from active *typhoid* infection his titer of typhoid agglutination will exhibit the usual rise and subsequent regular fall seen in non-inoculated subjects, but starting from and returning toward the higher base line of inoculated persons.
- (b) If the individual is suffering from active *paratyphoid* infection one of three things may occur as regards his *typhoid* agglutination titer, namely:
 1. No appreciable change may occur in the titer of typhoid agglutination.
 2. A relatively slight rise may occur followed by a fall toward the former level.
 3. A marked rise may occur synchronous with the rise in paratyphoid agglutination titer, and subsequently followed by the usual fall toward the former level.

¹ Lancet, London, 1916, 1, 241.

² Lancet, London, April 8 and September 2, 1916; March 10, 1917.

³ Lancet, London, 1916, 2, 546.

⁴ Lancet, London, 1917, 1, 263.

⁵ Deutsch. med. Wchn., 1916, 42, 1280.

⁶ Lancet, London, 1918, 1, 593.

Meanwhile the titer of *paratyphoid* agglutination runs the normal course of rapid rise to a maximum (usually exceeding the maximum typhoid titer), followed by a fall, at first rapid and then slower, as already described for typhoid subjects, and falling *below* the persistent base line of typhoid agglutination of inoculated persons.

In the case of **mixed infections**, whether in inoculated or non-inoculated persons, the agglutinin curves for the different infecting organisms are usually not synchronous, and they pursue their ordinary course independently of each other.

Agglutination in Bacillary Dysentery.—In *dysentery* the agglutination reaction with the serum of patients shows great variability. In spite of the presence of bacilli in the feces the reaction is sometimes absent, often disappears rapidly during convalescence, and rarely is as high as in typhoid fever. The tests should always be performed with both the "Flexner" and "Shiga" types of bacilli, as the two do not possess identical agglutinable properties, and either may be the cause of infection in a given case. The absence of the reaction does not exclude a dysenteric infection.

Martin, Hartley, and Williams¹ tested the serum from 151 cases of dysentery from whose stools dysentery bacilli had been isolated, and found an increase of agglutinin in slightly less than 40 per cent. of those infected with the Flexner-Y group of bacilli. In all cases infected with the Shiga strain, however, marked agglutination was observed, indicating that the agglutination test possesses most value in these infections. Ritchie² states that complete agglutination of *Bacillus dysenteriae* (Shiga) in dilution of 1 : 64 or higher is diagnostic, but with the Flexner strains agglutination should be complete in dilutions as high as 1 : 128 to be of diagnostic import. Friedman and Steinbock,³ Soldin,⁴ Dunner,⁵ Ledingham and Penfold⁶ have found agglutination tests of diagnostic value in infections among soldiers in the recent war.

Agglutination in Glanders.—In veterinary practice agglutination reactions are of value in the diagnosis of *glanders*, infected horses reacting in some instances to dilutions as high as 1 : 2000. For diagnostic purposes the agglutination test in glanders must be in dilutions higher than 1 : 800. A positive reaction in dilutions of 1 : 1000 is regarded as suggestive, and is controlled by a complement-fixation test; agglutination in dilutions of 1 : 1500 practically always indicates an infection. The complement-fixation test, however, is a better diagnostic reaction.

Povitsky⁷ has recently described an improved method for the preparation of the bacterial suspension and conduct of the test.

In the diagnosis of glanders among human beings a positive reaction in dilution of 1 : 100 or higher is considered positive, normal serum not reacting above 1 : 50.

Agglutination in Typhus Fever.—**Weil-Felix reaction:** In 1915 Weil and Felix⁸ isolated from the urine of typhus fever patients two strains of *Bacillus proteus vulgaris* which they found were agglutinated by the sera of individuals suffering with this disease. Later they succeeded in isolating a third strain (X 19) which proved more susceptible and has been extensively employed in agglutination tests. Friedberger⁹ believed that this bacillus may be the etiologic agent of typhus fever, but subsequent investigations by Land-

¹ Brit. Med. Jour., 1918, 1, 642.

² Lancet, London, 1916, 1, 245.

³ Deutsch. med. Wchn., 1915, 42, 213.

⁴ Deutsch. med. Wchn., 1915, 41, 845.

⁵ Berl. klin. Wchn., 1915, 52, 1177.

⁶ Brit. Med. Jour., 1915, 1, 37.

⁷ Jour. Immunology, 1918, 3, 463.

⁸ Wien. klin. Wchn., 1916, 29, 33, 927.

⁹ Deutsch. med. Wchn., 1917, xliii, Nos. 42-44.

steiner and Hausmann,¹ Doerr and Pick,² Mollers and Wolff³ have not supported this claim. However, the bacillus is agglutinated by the sera of the majority of individuals with typhus fever according to the reports of Ribeyo,⁴ Cancik,⁵ Dietrich,⁶ Dienes,⁷ Sacquepee,⁸ Braun,⁹ Jacobitz,¹⁰ Oettinger,¹¹ Schultz,¹² and others.

In applying the agglutination test for diagnosis the dilutions should range from 0 : 20 upward; agglutinating at 1 : 20 is regarded as suspicious. In typical cases agglutination may occur in dilutions as high as 1 : 250 to 1 : 1000. Agglutinins are first detected about the sixth day of the disease and rapidly disappear after the crisis.

Not all strains of *Bacillus proteus* are suitable for this reaction; indeed, the majority are unsuitable and laboratories conducting the test should secure strain X 19 for the work. Furthermore, this bacillus has been found in only a small percentage of cases of typhus fever. It is highly probable that the virus of the disease lowers resistance to *Bacillus proteus* and other bacilli of the colon group normally inhabiting the gastro-intestinal tract, thereby favoring antibody production. Analogous conditions are seen in scarlet fever favoring the streptococcus and poliomyelitis favoring various micrococci. As early as 1909 Wilson¹³ showed that heterologous agglutinins for colon and other intestinal bacteria were produced during typhus fever.

As the subject stands today it would appear that during typhus fever there may be the production of agglutinins for *Bacillus proteus* and other bacteria, but especially for strain X 19 of *B. proteus*, and that the agglutination test with this organism yields reactions of diagnostic value. Wolff¹⁴ has recently subscribed to Epstein's theory that *B. proteus vulgaris* originating in the intestinal tract undergoes, through symbiosis with the typhus virus, a change in its internal structure and becomes agglutinable by the serum in typhus fever. The reaction is not specific and requires more investigation for establishing its exact status.

AGGLUTINATION IN OTHER DISEASES

In *cholera* the agglutination test has so far proved of doubtful aid in establishing a diagnosis of the disease. However, for the purpose of recognizing bacilli isolated from the feces of suspicious cases the reaction with known immune serum is of great value.

In *cerebrospinal meningitis* the agglutination occurs within an hour in dilutions of 1 : 10. It is seldom that the patient's serum agglutinates in a dilution higher than 1 : 50.

In *tuberculosis* the agglutination reaction is regarded as having little or no value as a diagnostic procedure. Koch recommended the agglutination test for the estimation of the degree of immunity conferred by tuberculin treatment. As pointed out elsewhere, agglutinins have apparently no anti-

¹ Med. Klin., 1918, 14, 515.

² Wien. klin. Wchn., 1918, 31, 820.

³ Deutsch. med. Wchn., 1919, No. 13, xlv.

⁴ Cronica Med., 1919, 36, 75.

⁵ Wien. klin. Wchn., 1916, 29, 1552.

⁶ Deutsch. med. Wchn., 1916, 42, 1570.

⁷ Deutsch. med. Wchn., 1919, 45, 14; Ztschr. f. Immunitätsf., orig., 1919, 25, 447.

⁸ Bull. d. l. Soc. Med. d. Hôp., 1919, 43, 151.

⁹ Centralbl. f. Bakteriöl., orig., 1918, 8, 20, 475.

¹⁰ Centralbl. f. Bakteriöl., orig., 1918, 81, 251.

¹¹ Centralbl. f. Bakteriöl., orig., 1918, 80, 304.

¹² Amer. Jour. Med. Sci., 1921, clxi, 78.

¹³ Jour. Hyg., 1910, ix, 316; *ibid.*, 1910, x, 155; *ibid.*, 1920, 19, 115.

¹⁴ Berl. klin. Wchn., 1920, 57, 834.

microbic influence, but, as with typhoid vaccination, may indicate the degree of reaction and the presence of other antibodies.

Many strains of tubercle bacilli are almost non-agglutinable. The preparation of a homogeneous emulsion is not easily made, and the results are likely to be confusing and contradictory. Recently, however, Larson, Nelson, and Chang¹ have described a method for preparing antigen by subjecting tubercle bacilli to the influence of carbon dioxide under high pressure, and believe that the test possesses diagnostic value.

In *plague* the agglutination reaction becomes quite marked about the ninth day of the disease—too late, however, to be of much practical value in diagnosis. It is occasionally useful, however, for deciding whether a patient in the convalescent stage has really suffered from the disease.

In *Malta fever* the agglutination reaction is of considerable value in making the diagnosis. Salvatore² states that agglutination at 1 : 40 may be regarded as specific for *Micrococcus melitensis* if the typhoid reaction is negative. Agglutination should be positive in dilutions of 1 : 100 to be of significance in diagnosis among goats and other cattle.

In *pneumonia* the reaction is of value in rapidly differentiating pneumococci and as an aid in specific serum therapy (see page 286).

In *syphilis* agglutinins for *Treponema pallidum* by the sera of rabbits injected with a living and heat-killed culture furnished by Noguchi were first described by Kolmer³; Nakano,⁴ Kissmeyer,⁵ and Zinsser and Hopkins⁶ have also described the agglutination of culture pallidum by immune serum. Kolmer, Broadwell, and Matsunami⁷ found that equal parts of normal human serum and pallidum culture furnished by Zinsser may result in partial agglutination, whereas with sera of syphilitics in the later stages agglutination in dilutions of 1 : 5 and higher occurred with about 84 per cent. of sera, but that further studies are necessary to establish the practical value of agglutination in the diagnosis of syphilis.

In *pertussis* agglutination tests have been found of some value in clinical diagnosis by Wollstein,⁸ Fränkel,⁹ Seiffert,¹⁰ and Arnheim; Bordet¹¹ finds it of value only when the micro-organism is freshly isolated from human sputum and grown on rich blood medium. According to Povitsky¹² and Worth¹³ a dilution of serum not less than 1 : 200 is necessary for a practical positive diagnosis of pertussis, as normal human serum may agglutinate in dilutions up to 1 : 100.

In *sporotrichosis* agglutination reactions have been described by Widal,¹⁴ Davis,¹⁵ and others and are believed to possess diagnostic value, agglutination occurring in dilutions of from 1 : 300 to 1 : 800 in an hour; Moore and Davis¹⁶ have recently reported favorably upon the specificity and diagnostic value of agglutination and complement-fixation reactions in sporo-

¹ Proc. Soc. Exper. Biol. and Med., 1922, 19, 359.

² Policlinico, 1914, 20.

³ Jour. Exper. Med., 1913, xviii, 18.

⁴ Archiv. Dermat. u. Syph., 1913, cxvi, 265.

⁵ Deutsch. med. Wchn., 1915, xli, 306.

⁶ Jour. Exper. Med., 1915, xxi, 576.

⁷ Jour. Exper. Med., 1916, xxiv, 333.

⁸ Jour. Exper. Med., 1909, xi, 41.

⁹ Münch. med. Wchn., 1908, lv, 1683.

¹⁰ Münch. med. Wchn., 1909, 1561.

¹¹ Berl. klin. Wchn., 1908, xlv, 1453.

¹² Centralbl. f. Bakteriolog., orig., 1912, lxvi, 276.

¹³ Archiv. Int. Med., 1916, xvii, 279.

¹⁴ Ann. d. l'Inst. Pasteur, 1911, 24.

¹⁵ Jour. Infect. Dis., 1913, 12, 140.

¹⁶ Jour. Infect. Dis., 1918, 23, 252.

trichosis and point out that these tests are of much less value in the diagnosis of blastomycosis, which is a closely related disease.

Agglutination reactions have also been reported in *leprosy* by Harris and Lanford,¹ Nakajo,² and others. In *dourine* and other trypanosome infections by Mattes³ and Wehrbein,⁴ and in *malaria* by Biglieri.⁵

The "group agglutinins" constitute a source of difficulty in making the differentiation among the numerous members of a group of micro-organisms, but if a highly potent agglutinating serum is used and the test is carried to the point of determining the highest dilution that will agglutinate the bacteria, it will in most cases be possible to differentiate the variously allied micro-organisms by this test.

In conducting these reactions it is best to use a macroscopic method, and the agglutinating serum used must have been previously titrated against an easily agglutinable and known strain of the micro-organism in question.

In this connection it is well to remember that freshly isolated cultures of a micro-organism may be not at all or but very slightly agglutinable. Thus, colonies of typhoid bacilli found in feces or in an abscess may, if picked from a plate, resist agglutination until subcultured several times in artificial media.

The agglutination test has great value as a mode of differentiating among the members of the typhoid-colon group of bacilli. In the diagnosis of cholera, suspicious bacilli isolated from the feces may be tested with a known cholera immune serum, and the bacteriologic diagnosis thus be greatly facilitated. The test also has some value in making a biologic differentiation between meningococci and gonococci, and also between other groups of bacteria.

Production of Immune Agglutinins.—In the preparation of immune sera for diagnostic agglutination reactions, rabbits are generally employed. The micro-organisms may be injected intravenously, intraperitoneally, or subcutaneously; as shown by McFarland,⁶ working with *Bacillus coli*, the route of injection has little or no influence upon agglutinin production. Intravenous injections usually produce agglutinins more quickly and in larger amounts than other routes.

Due care must be exercised against the injection of toxic amounts of culture. As shown by Perry and Kolmer,⁷ the administration of living cultures of typhoid bacilli produce most agglutinin. It is a good plan to start immunization with heat-killed suspensions, and as antibodies are produced living cultures may be used. The injections should be at intervals of five to seven days and the blood tested at intervals; final bleeding may be done about seven to nine days after the last injection.

1. Use forty-eight-hour agar cultures of the organism, such as *Bacillus typhosus*, *Spirillum cholerae*, etc. Bouillon cultures may be used, but are not recommended on account of the various other constituents present in the medium.

2. With a sterilized 4-mm. platinum loop remove 1 loopful of culture, and rub up in 2 c.c. of sterile salt solution in a small test-tube until a homogeneous emulsion is secured.

3. Heat the emulsion for thirty minutes at 60° C. in a water-bath.

4. Inject intravenously.

¹ Jour. Med. Research, 1916, 34, 157.

² Jour. Infect. Dis., 1915, 17, 388.

³ Centralbl. f. Bakteriol., 1912, 65, 538.

⁴ Jour. Infect. Dis., 1915, 16, 461.

⁵ Wien. klin. Wchn., 1915, 28, 1049.

⁶ Ztschr. f. Immunitätsf., orig., 1911, 9, 451.

⁷ Jour. Immunology, 1918, 3, 247.

5. Give four more injections at intervals of five days as follows:

Second dose: 2 loopfuls in 2 c.c. NaCl solution, heated.

Third dose: 4 loopfuls in 2 c.c. NaCl solution, heated.

Fourth dose: 6 loopfuls in 2 c.c. NaCl solution, heated.

Fifth dose: 1 agar slant in 4 c.c. NaCl solution, heated.

Sixth dose: 1 agar slant in 4 c.c. NaCl solution, *unheated*.

6. One week after the last injection has been made the blood is tested, and if found of satisfactory titer, the animal is killed and the serum secured. If the titer is found too low, one or more additional injections are given.

Intraperitoneal Method (Rabbit).—1. Same as the preceding, excepting that larger doses are given.

First dose: 2 loopfuls in 4 c.c. NaCl solution, heated.

Second dose: 4 loopfuls in 4 c.c. NaCl solution, heated.

Third dose: 6 loopfuls in 4 c.c. NaCl solution, heated.

Fourth dose: 1 agar slant in 5 c.c. NaCl solution, heated.

Fifth dose: 1 agar slant in 5 c.c. NaCl solution, *unheated*.

Sixth dose: 1 agar slant in 5 c.c. NaCl solution, *unheated*.

2. The blood is tested one week after the last injection has been made.

TECHNIC OF BACTERIAL AGGLUTINATION REACTIONS

Two methods may be employed:

1. The *microscopic method* which is generally employed where the Gruber-Widal reaction for typhoid fever has been employed as the reaction is quickly done and requires but a small amount of blood.

This test is usually performed with serum separated from the clot and in various dilutions (wet method). The test may also be performed with dried blood (dry method), the agglutinins being preserved and redissolved with a diluent. The technic of the latter method is very simple. The blood is easily collected and may be sent for long distances, and for these reasons the method has been adopted by many boards of health.

2. The *macroscopic method* is that generally preferred if sufficient blood is on hand, and is the method of choice in scientific research. Absorption tests must be performed with the macroscopic technic.

Macroscopic methods were first introduced by Wright¹ in 1897 as a substitute for Widal's microscopic methods. Madsen and Jorgensen,² however, were first to devise an accurate quantitative method, although their technic has been long since superseded by simpler and equally accurate methods. Possibly the best and most generally useful method for accuracy, precision, simplicity, and safety is that devised by Dreyer,³ which has proved so valuable for diagnostic purposes during the recent war.

Advantages and Disadvantages of Microscopic Methods.—The *advantages* are: (1) The reactions may be read within an hour after the tests are set up, thereby yielding quick results; (2) only small amounts of serum are required.

The *disadvantages* are: (1) The living bacterial emulsions or broth cultures employed are likely to vary greatly in density from day to day and thereby reduce the accuracy of the tests. (2) Cultures are likely to vary in age and sensitiveness to agglutination which greatly influence accuracy.

¹ Brit. Med. Jour., 1897, 1, 139.

² Festschrift ved Indv. a. Stat. Seruminst., Copenhagen, 1902.

³ Hospitalst., Copenhagen, 1906. Brit. Med. Jour., 1904, 2, 564. Jour. Path. and Bacteriol., 1909, 13, 332; *ibid.*, 1906, 1.

(3) Slight differences in the constitution of the culture medium employed may influence the accuracy of agglutination tests with living cultures. (4) It is not usually possible to conduct the tests with as great quantitative accuracy as the macroscopic test. (5) Weakly positive indefinite reactions are more difficult to read, and more subject to error in interpretation than macroscopic reactions.

Advantages and Disadvantages of Macroscopic Methods.—The advantages are: (1) The technic permits the use of formalized cultures prepared after the method of Dreyer, which are highly sensitive to agglutination and of uniform density. (2) The bacterial emulsion prepared by this method is sterile and thereby safe. (3) The emulsion may be kept under suitable conditions for a year or longer and is always ready for use. (4) The method is more accurate and very simple. (5) The readings are quite sharp and definite and readily made. (6) *Most important of all, repeated agglutination tests with the serum of the same individual may be rendered more uniform than is usual with microscopic tests; this is of particular value in the diagnosis of typhoid and paratyphoid fevers among individuals who have received vaccines.*

The only *disadvantage* is: The longer time required before readings may be made (two hours for typhoid reactions; four hours for dysentery; but sharper readings require twenty-four hours). An additional disadvantage for Board of Health laboratories is the difficulty in securing a sufficient supply of blood for serum.

I am convinced that the macroscopic technic is the method of choice and especially in the diagnosis of intestinal infections among vaccinated individuals. The work of Dreyer and his associates has proved the superlative merits of the method not only for research work but for routine examinations as well.

TECHNIC OF THE MICROSCOPIC AGGLUTINATION TEST (WET METHOD).—THE WIDAL REACTION IN TYPHOID FEVER

1. The bacterial emulsion should be prepared of young cultures, should be homogeneous and free from clumps, and of such density as to furnish a sufficient number of micro-organisms to give the reaction (Fig. 98).

For the ordinary microscopic Widal test, eighteen to twenty-four-hour bouillon cultures of *Bacillus typhosus*, *B. coli*, and *B. paratyphosus* may be employed. An old laboratory culture—one that is known to be agglutinable—should be used. Broth cultures should be cultivated at a temperature lower than body heat in order that long motile forms may be secured. During the summer and early autumn months the culture can be grown at room temperature; during the winter, on top of the incubator.

Thick cultures are unsatisfactory for making the microscopic test, as there is always some false clumping and motility is not well marked (Fig. 99).

When these tests are done routinely, it is good practice to subculture in broth every day in order that a satisfactory culture may always be on hand. When performed at irregular intervals, a broth culture can be prepared from a stock agar culture and the test performed twenty-four hours later.

Emulsions may be prepared of young cultures on solid media by removing portions of the growth with a platinum loop and emulsifying in a diluent, such as normal salt solution or broth. This may be performed by placing the diluent in a test-tube and rubbing the loop over the glass just at the margin of the fluid, the bacteria being gradually emulsified and floated into the diluent. The emulsion is gently shaken and removed to a

second tube, when unresolved bacterial clumps will sink to the bottom. In other cases the emulsion may be centrifuged for a short time or filtered through sterile filter-paper. Sufficient salt solution is added to give the emulsion a density equal to that of a rich twenty-four-hour bouillon culture.

2. Sufficient blood may be obtained by pricking a finger and filling a Wright capsule; or 0.5 to 1 c.c. may be collected in a small test-tube.

After standing a few hours the serum may be pipeted off the clot; or immediately after coagulation the clot may be broken up and the serum secured by centrifuging.

The serum should be fresh, clear, and free from corpuscles.

Liebermann and Acel¹ have described a method of collecting 2 drops of blood in 1 c.c. of distilled water which hemolyses the red blood-corpuscles and gives an approximate dilution in 1 : 20.

3. Dilute the patient's serum by placing 1 drop from a capillary pipet in a small watch-glass and adding 19 drops of normal salt solution. This gives a dilution of 1 : 20. Mix thoroughly.

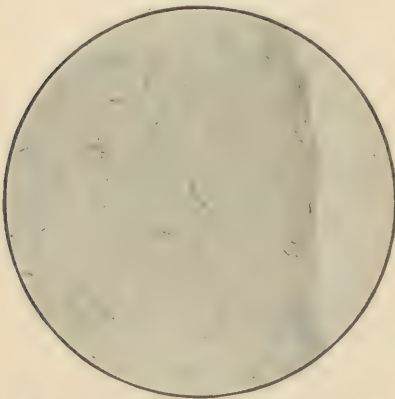


FIG. 98.—A SATISFACTORY CULTURE FOR THE MICROSCOPIC AGGLUTINATION REACTION. $\times 430$.

This shows a satisfactory culture of the proper density and free of clumps of bacilli. (Twenty-four-hour culture of *Bacillus typhosus* grown at room temperature.)

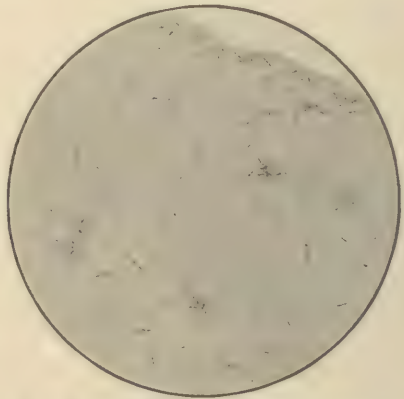


FIG. 99.—AN UNSATISFACTORY CULTURE FOR THE MICROSCOPIC AGGLUTINATION REACTION. $\times 430$.

The culture is rather too dense and shows considerable spontaneous or false agglutination of the bacilli. (Twenty-four-hour culture of *Bacillus typhosus* grown at 37° C.)

Because normal agglutinins may be active in dilutions as high as 1 : 20, for diagnostic tests in typhoid fever the serum should not be diluted lower than 1 : 10 (final dilution 1 : 20). For routine work dilutions of 1 : 50 and 1 : 100 are well adapted for the microscopic test. Dilutions of 1 : 40 and 1 : 80 are readily made and are equally useful.

4. With a 3 or 4 mm. platinum loop place a drop on a clean cover-glass that is sufficiently thin to permit the use of an oil-immersion lens. The loop is better than a capillary pipet because the drop it gives is smaller, and when it is later diluted with an equal quantity of bacterial emulsion it is not too large and is easily manipulated.

5. With the same sterilized platinum loop add 1 loopful of a twenty-four-hour broth culture of *Bacillus typhosus* to the drop of diluted serum on the cover-glass. Mix gently and without spreading the drop. This gives a final dilution of 1 : 40.

¹ Deutsch. med. Wchn., 1914, 40, 2057.

6. Edge a hanging-drop slide with vaselin, and invert the cover-glass slide over the hollow portion in such a manner that the drop will be suspended in its center. Care must be exercised not to spread the drop, for if this occurs and the fluid flows around the margins of the chamber a new preparation must be made. Inspect the slide, and add vaselin, if necessary, until it is sealed tightly. By means of a grease pencil label the slide with the name of the patient, the dilution, and the time when the preparation was made.

7. Place 5 drops of serum dilution 1 : 20 in a second watch-glass, and add an equal quantity of normal salt solution. Mix well. This gives a dilution of 1 : 40.

8. Prepare a second slide by mixing a loopful of this dilution with an equal sized loopful of culture. Mix gently. This gives a final dilution of 1 : 80. Mark the slide with the name, dilution, and the time.

9. Prepare a third slide by placing a loopful of culture on a cover-glass and invert over a concave slide to which vaselin has been applied in the usual manner. This is the culture control. Label the slide.

10. Place the slides in a dark place at room temperature and examine at the end of an hour with the $\frac{1}{8}$ or oil-immersion lens.

(a) First inspect the control. The bacilli should not be clumped, but should be motile, and preferably in the form of long slender rods (see Fig. 98).

(b) Examine the 1 : 40 and 1 : 80 dilution preparations: a *positive reaction* is indicated by loss of motility and definite clumping (Fig. 100). A few free motile bacilli may be seen, or a clump may be seen to move, owing to the efforts of the bacilli to break away. A *doubtful reaction* is indicated by a partial loss of motility and a few indefinite clumps. A *negative reaction* is indicated when there is no loss in motility or no clumping, or when the reactions resemble the control to which no serum has been added. In reporting upon agglutination tests always state the time at which the test was made and the dilution used.

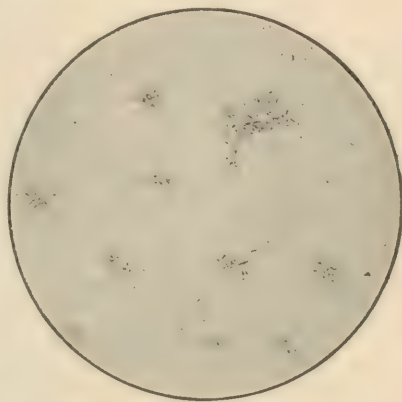


FIG. 100.—A POSITIVE AGGLUTINATION (WIDAL) REACTION IN TYPHOID FEVER. $\times 430$. Serum from a patient ill about twenty-two days; a 1 : 100 dilution at the end of one hour.

A 1 : 20 and a 1 : 40 dilution may be prepared and examined at the end of half an hour. Prompt agglutination is found practically only in typhoid fever.

Dilutions may be conveniently prepared by drawing the serum up to the mark 0.5 in the white corpuscle pipet, and the *distilled water* up to the mark 11. Mix well. This gives a dilution of 1 : 20. One loopful of this diluted serum and 1 loopful of bouillon culture of the micro-organism to be tested give a dilution of 1 : 40. One loopful of the 1 : 20 diluted serum and 3 loopfuls of the culture give a dilution of 1 : 80. Having mixed the diluted serum and the bacterial suspension on a cover-glass, prepare the cultures on the vaselined concave slides in the usual manner.

Precautions.—In bacteriologic technic due care should be observed to avoid contamination and possible infection when working with living cultures.

(a) Agglutinated bacteria are not necessarily dead, and hanging-drop preparations, test-tubes, etc., should be immersed in 1 per cent. formalin before cleansing.

(b) The working table or desk and the hands should be washed with a solution of lysol or 1 per cent. formalin after the reactions have been made and the work completed.

(c) Early in typhoid fever the bacillus may be present in the blood, and consequently due care should be exercised in handling it, in diluting the serum, and in the disposal of the clot.

(d) Great care must also be exercised against the error of falsely positive reactions due to the use of *spontaneously agglutinating organisms*. For this reason a control employing normal serum or simple saline solution should always be employed. Arkwright¹ has recently shown that suspensions of these organisms in 0.42 to 0.1 per cent. saline solutions may prevent spontaneous agglutination.

THE MICROSCOPIC AGGLUTINATION TEST (DRY METHOD)

1. The culture is prepared as described above.

2. Blood is secured by pricking the finger or lobe of the ear and collecting a few drops of blood upon aluminum foil, on a clean glass slide, or on partially glazed paper. *The blood must not be heated to hasten drying*, or agglutinins may be destroyed. Smears on aluminum foil and on glass slides are to be preferred to those on paper, as the blood can be moistened and portions removed without the likelihood of transferring extraneous material, such as paper fiber. While there are certain objections to this method to be pointed out later, yet practical experience has demonstrated its value, as the serum does not readily deteriorate or become contaminated with bacteria, and the ease with which blood may be collected and mailed recommends the process for board of health laboratories.

3. Place a loopful of a twenty-four-hour bouillon culture of *Bacillus typhosus* in the center of a clean cover-glass.

4. Moisten the dried blood which has been collected on aluminum foil, glass slide, or paper with a loopful of normal salt solution. (A second and smaller loop may be used for this purpose.) Gently rub up the dried blood and transfer a sufficient amount to the drop of culture on the cover-glass until, when thoroughly mixed, it presents a *delicate orange tint* (Fig. 101). Avoid transferring too much debris with the solution of blood, especially if the blood has been collected on paper. It is good practice to mix the culture and solution of blood with the cover-glass held over a white surface, in order that the color may readily be observed.

5. Having made the mixture on the cover-glass, invert it over a vaselined concave slide, label, and stand aside for an hour.

6. Prepare the culture control in the usual manner and label.

7. Examine at the end of an hour with the $\frac{1}{6}$ or oil-immersion lens. If minute fragments of fiber, etc., have been transferred, due allowance for false agglutination for these should be made. Otherwise the readings are made in exactly the same manner as in the "wet" method.

8. Accurate dilutions are not possible with this technic. Satisfactory results are dependent largely upon the color; a faint orange tint of the suspension is desirable, and probably represents a dilution of about 1 : 40. This method, however, is very simple, and when carefully performed yields results in the practical serum diagnosis of typhoid fever almost as satisfactory as the serum-dilution method.

¹ Jour. Path. and Bacteriol., 1921, 24, 36.

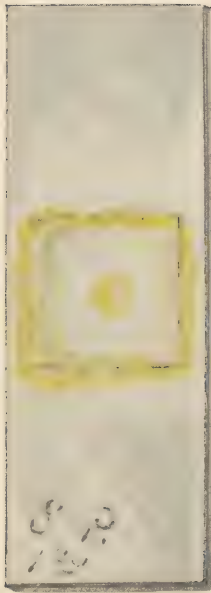


FIG. 101.—MICROSCOPIC AGGLUTINATION TEST WITH DRIED BLOOD.

Shows the proper color of the suspended drop of typhoid culture when the solution of dried blood has been added. The tinge should be *light orange* or *yellow*, and a shade lighter than ordinary vaselin used in sealing the preparation.

It is possible, however, to work with known approximate dilutions by the dried blood method if a good chemical balance is available. Blood must be collected on aluminum foil or glass, and is then scraped off and weighed. To each 5 mg. of dried blood 0.5 c.c. of salt solution is added which equals a dilution of 1 : 25 of whole blood or 1 : 100 of dried blood (Wesbrook). After permitting the mixture to stand for half an hour it is centrifuged for a short time. To 1 drop of the dilution thus obtained 1 drop of culture is added, which gives a final dilution of about 1 : 50. At the end of an hour it is examined. Higher dilutions can be prepared from this stock dilution at the will of the operator.

MACROSCOPIC AGGLUTINATION TEST

The Bacterial Suspension.—Living cultures may be employed by cultivating the bacterium in broth or removing and emulsifying cultures from solid medium in physiologic saline solution as described under the microscopic method.

The suspension should be free of macroscopic clumps and of a density equal to tubes 5 or 6 of McFarland's nephelometer (see page 196). Furthermore, the suspension must not show spontaneous agglutination.

To emulsify a culture of the plague bacillus or any other micro-organism that displays a strong tendency to undergo "spontaneous" agglutination, distilled water or 1 : 1000 salt solution should be used.

In the case of a culture of tubercle bacillus, the growth can be resolved into its elements by prolonged trituration in normal salt solution, and any residue or unresolved clumps removed by centrifugalization. A less laborious and dangerous method is to use the tubercle powder of Koch, which is obtained by reducing dried tubercle cultures to a fine powder by machinery. The powder may be made up into a suitable suspension by rubbing it in a mortar with normal salt solution.

When it is necessary to work with highly dangerous micro-organisms, or to operate from day to day with the same bacterial suspension, one may employ suspensions that have been heated for one hour to 60° C., or suspensions in salt solution to which 1 per cent. formalin has been added. These will keep well in the refrigerator, but the sediment of dead bacteria must be well shaken before it is used.

Nobel¹ has described the following method for preparing a suspension of anthrax bacilli which are so prone to produce spontaneous agglutination:

"The cultures are transplanted daily for ten days on plain agar and incubated at 42.5° C., until a sporeless and very vigorous growth is obtained. Each strain is then planted on plain agar in quart whisky flasks and incubated for twelve hours at 42.5° C. The growths are washed off in physiologic salt solution containing 0.5 per cent. formalin (about 100 c.c. to a flask). The suspensions are shaken in a mechanical shaker for forty-eight hours. After standing for several days and being tested for sterility, equal parts of each suspension are mixed in a cylinder; shaken for twenty-four hours and allowed to stand over night. The larger clumps settle out leaving a homogeneous suspension above. This upper portion is poured off and filtered several times through four thicknesses of sterile cheese-cloth. The suspension is then diluted with physiologic salt solution plus 0.5 per cent. formalin to a density corresponding to a suspension of *Bacillus typhosus* containing 2,000,000,000 bacteria per cubic centimeter. A suspension of *B. anthracis* so prepared is perfectly homogeneous, stands up for at least forty-eight hours at 37° C., and shows no spontaneous agglutination."

¹ Jour. Immunology, 1919, 4, 105.

Dreyer Method.—As previously stated, Dreyer has found suspensions of typhoid, paratyphoid, dysentery, and other intestinal bacilli best prepared by using broth cultures sterilized and preserved with 0.1 per cent. formalin. This method has been warmly endorsed by numerous English workers; Sands,¹ working in my laboratory, has also found formalized emulsions best for the typhoid agglutinin reaction.

Dreyer has described the preparation of formalized suspensions as follows²:

(a) The bacillus (*B. typhosus*, *B. paratyphosus*, etc.) is grown for twenty-four hours at 37° C. in ordinary *veal* peptone bouillon in large Erlenmeyer flasks partly filled (1 liter of bouillon in a 1½-liter flask).

(b) Before use the flasks of bouillon are sterilized in the autoclave at 115° C. for *not more* than fifteen minutes, and are then tested for sterility by incubation at 37° C. for forty-eight hours.

(c) They are inoculated with a few drops each from a twenty- to twenty-four-hour old bouillon culture of the bacillus (*B. typhosus* or *B. paratyphosus*, etc.).

(d) The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

(e) At the end of twenty-two to twenty-four hours' growth at 37° C. the flasks are well shaken, and to each is added 0.1 per cent. (1 c.c. per liter) of commercial (40 per cent.) formalin. They are again shaken and placed in a cold chamber in the dark at about 2° C.

(f) At intervals on the same day and on subsequent days for four or five days the flasks are again thoroughly shaken and *replaced at once in the cold chamber*.

(g) After three or four days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton-wool. Cultures so prepared are put into sterile bottles with rubber stoppers and kept in a cold and dark place.

The advantages of this method may be summarized as follows:

(a) The bacteria are dead and fixed and their use devoid of danger.

(b) The suspensions contain no excess of antiseptics which may be detrimental to agglutination.

(c) There is either no loss of agglutinability at all or but a transitory loss of slight degree (*B. dysenteriae*).

• (d) The suspensions can be kept for six months or longer without change.

By means of this method Dreyer has been able to standardize the agglutinin test for typhoid, paratyphoid, and other intestinal infections; he has shown quite conclusively that consistent results are only possible by conducting tests with suspensions of bacteria of uniform agglutinability and density.

The suspension should always be practically transparent. It is stated by the Oxford Standard Laboratory (where Dreyer and his associates prepared standard emulsions for use in English laboratories and especially for army laboratories during the war) that "the growth of a mold in a bottle does not affect the agglutinability of the culture. If the mold be fished out and a drop or two of chloroform be added to the fluid to prevent further growth the culture is as good as ever. Bacterial growths in the cultures

¹ Jour. Immunology, 1920, 5, 97.

² Jour. Path. and Bacteriol., 1909, 13, 331.

also occur, but are rare and almost invariably the result of careless handling." Krumbhaar and Smith¹ found that even with careful handling contamination was apt to occur and they have advised removing from the stock bottle an amount sufficient for the work at hand, discarding any that is left over. I believe that more formalin may be added to lessen the risks of contamination without injury to the suspension. In my experiments 1 per cent. *neutral* formalin proved very satisfactory (10 c.c. neutral formalin per liter of culture).

Serum.—Sufficient blood for the macroscopic test may be obtained by pricking the finger and filling a Wright capsule or collecting about 1 c.c. in a small test-tube. The serum may be allowed to separate or may be obtained at once by breaking up the clot and centrifuging. It should be free of corpuscles.

The Test.—All dilutions and measurements are to be made with accurately graduated 1 c.c. pipets (dry and preferably sterile).

Water instead of physiologic saline solution is employed for making the dilutions. Dreyer has recommended the use of distilled water and Krumbhaar and Smith have found the tests superior to those conducted with saline solution.

1. Place a row of seven small test-tubes (10 x 1 cm.) in a test-tube rack and add 1 c.c. of sterile distilled water to each.

2. Dilute the serum 1 : 5 in the first tube as follows: 0.2 c.c. serum plus 0.8 c.c. water. This now gives in this tube 2 c.c. of a dilution of 1 : 10. Mix well with the pipet.

3. Place 1 c.c. of the serum from tube 1 into tube 2. Mix well, and place 1 c.c. of the mixture from tube 2 into tube 3, and so on. When the sixth tube has been reached discard 1 c.c., as no serum is to be added to the seventh tube which is the culture control; *i. e.*, it will contain water plus bacterial emulsion.

4. Add 1 c.c. of bacterial emulsion to each tube which doubles the serum dilution in each. Tube 1 now contains a serum in a dilution of 1 : 20, acting on the bacteria; tube 2, one of 1 : 40; tube 3, one of 1 : 80; tube 4, one of 1 : 160; tube 5, one of 1 : 320; tube 6, one of 1 : 640. Tube 7, as just stated, contains the bacterial emulsion in water and is the culture control.

In determining the agglutination titer of a highly immune serum these dilutions may be continued to any degree.

5. On each tube the final dilution is marked with a wax pencil. The tubes are then shaken gently, stoppered with cotton plugs, and placed in the incubator at 37° C. or in a water-bath at 55° C. for two hours. The tubes are then allowed to remain at room temperature for a few hours, or in the refrigerator for twenty-four hours, after which readings are made.

When living cultures are employed the *method of Kolle and Pfeiffer* is very convenient, and may be safer than that of adding live cultures with a pipet. It is conducted as follows:

1. Make dilutions of serum as described.

2. Emulsify thoroughly a loopful (2 mg.) of culture from an eighteen- to twenty-four-hour-old agar culture in the first test-tube, repeating the process in the second tube, and so on through the series. In this method the serum dilutions are not doubled; thus in the foregoing series the dilutions would be 1 : 10, 1 : 20, 1 : 40, 1 : 80, 1 : 160, 1 : 320.

3. The tubes are gently shaken, labeled, plugged, and incubated as directed in the preceding method.

¹ Jour. Infect. Dis., 1918, 23, 126.

The Readings.—The culture control should show a uniform cloudiness, with no sediment or flakes, or at most a very slight precipitate that is readily

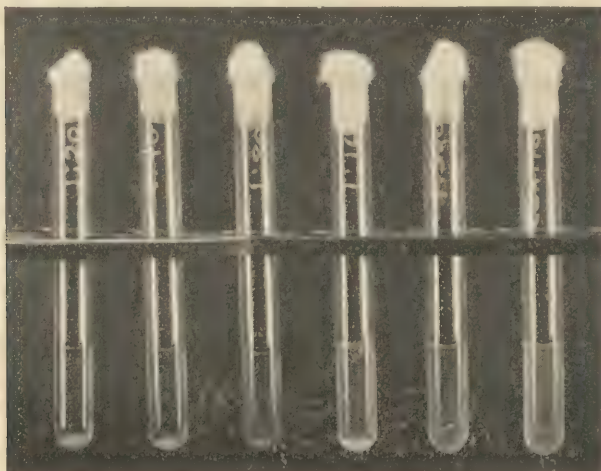


FIG. 102.—MACROSCOPIC AGGLUTINATION REACTION.

Serum of an individual who had received three injections of typhoid vaccine. This drawing was made twenty-four hours after the test was set up. The dilutions were: 1:20, 1:40, 1:80, 1:160 and 1:320; the sixth tube is the control.

broken up by gentle agitation. A positive reaction shows masses and clumps of bacteria adhering to the sides and bottom of the tube, which

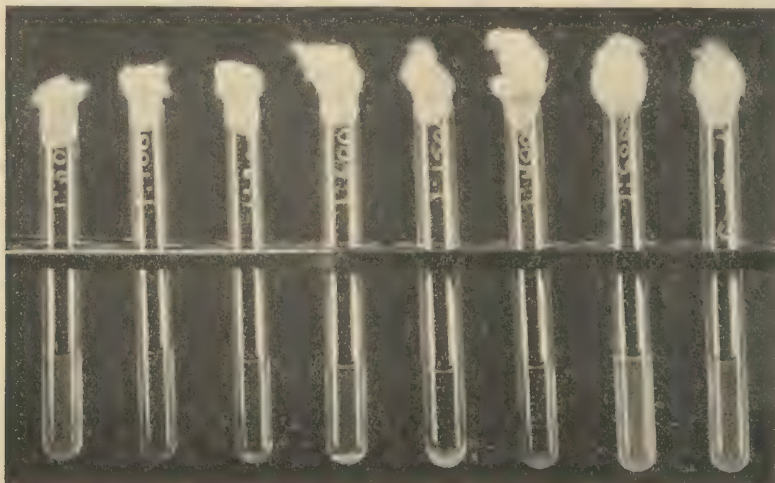


FIG. 103.—MACROSCOPIC AGGLUTINATION REACTION. SHOWS ACTION OF AGGLUTINOIDS (PRO-AGGLUTINATION).

Note absence of agglutination in dilution 1:50; agglutination beginning in 1:100, and fairly well marked to 1:4000 inclusive. Note uniform cloudiness of control. This reaction was set up with a typhoid immune serum over six months of age; the drawing was made after the tubes had been incubated two hours and placed in a refrigerator overnight.

are broken up with some difficulty (Fig. 102). The supernatant fluid should be clear. As dilutions become higher and the amount of contained agglu-

tinin correspondingly less, agglutination becomes less and less complete. There is less sediment, and the turbidity of the supernatant fluid is greater, until the negative tube closely resembles the culture control. A microscopic examination of a deposit will show that the bacilli point in all directions, whereas in a deposit of unagglutinated bacilli they lie horizontally side by side.

When *agglutinoids* are present, agglutination is absent or incomplete in the lower dilutions of serum, and complete in the tubes containing the higher dilutions. This is called **proagglutination** (Fig. 103).



FIG. 104.—AGGLUTINOSCOPE. (Altman.)

The test-tubes are arranged in the rack and viewed from below in the mirror. In this manner the smallest deposits are easily seen and compared with the control.

Readings are facilitated by the use of a special instrument known as the *agglutinoscope* (Fig. 104). The tubes are placed in a rack having numbered holes, and are viewed from beneath with the aid of a mirror. In this way one looks upward through the column of fluid, and secures a combined view of sediment and turbidity, and when examined with the culture control, fine and accurate readings may be made.

If the readings are made within a few hours after incubation it is well to use a hand glass of about 2 to 4 diameters of magnification. Krumbhaar and Smith found that this facilitated the readings of reactions conducted

by the Dreyer method. If the tubes are allowed to stand over night before the readings are made the naked eye is sufficiently accurate.

Hadley¹ has recently advocated the adoption of a standard method of reading and recording reactions; the general adoption of his method would render results reported from different laboratories more uniform.

Dreyer Standardized Agglutination Test.—1. The bacterial suspension is prepared as previously described consisting of broth cultures of strains selected for their high specificity, killed and preserved with 0.1 per cent. formalin. In England successive batches of standard agglutinable culture the relative sensitiveness to agglutination of the bacilli is indicated by a figure—the so-called *Reduction Factor*.

2. The test is conducted by the drop method, the use of Dreyer's pipets being preferable. These are made on the same lines as an ordinary dropping pipet (18.8 cm. in length; the end is drawn out to a length of 2.8 cm., with an external diameter of 0.235 cm. and a bore of 0.05 cm.).

3. The test-tubes are conically pointed at the lower end: They are 6 cm. long, 0.66 cm. in diameter; the lip is widened out to 1 cm.

4. In conducting a test for typhoid and paratyphoid agglutinins proceed as follows: If a test for typhoid agglutinins only is to be done but one row of tubes is required:

(a) Take a stand containing 15 agglutination tubes in 3 rows of 5 each, and a dilution tube.

(b) With the proper dropping pipet measure out into the dilution tube 54 drops of distilled water or normal saline solution (0.85 per cent. sodium chlorid in distilled water).

(c) Wash the pipet with distilled water.

(d) Dry out the pipet with successive quantities of absolute alcohol, followed by successive quantities of ether, and get rid of the ether.

(e) Take up the serum to be tested into the dried pipet. Measure out 6 drops of the serum into the dilution tube already containing the 54 drops of saline solution, thus obtaining a dilution of 1 in 10. Mix thoroughly.

Carefully wash out the pipet.

With the pipet measure out into each row of tubes as follows:

Number of Tube.	Drops of Normal Saline ² Solution.	Drops of Serum Dilution 1 in 10	
1	0	10	} To each tube in row 1 add 15 drops of <i>Bacillus typhosus</i> culture.
2	5	5	
3	8	2	
4	9	1	} To each tube in row 2 add 15 drops of <i>B. paratyphosus A</i> culture.
5	10	0	
			} To each tube in row 3 add 15 drops of <i>B. paratyphosus B</i> culture.

At each stage of the procedure the pipet is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantities of ether.

(f) Shake each tube thoroughly in order from right to left, *i. e.*, beginning each row with the highest dilution.

(g) Place the stand for two hours in a water-bath at 50° to 55° C. (*not* in dry air).

¹ Jour. Immunology, 1917, 2, 463.

² Or, preferably, distilled water.

In Tube 1 of each row the serum acts in a dilution of 1 in 25.

In Tube 2 of each row the serum acts in a dilution of 1 in 50.

In Tube 3 of each row the serum acts in a dilution of 1 in 125.

In Tube 4 of each row the serum acts in a dilution of 1 in 250.

Tube 5, containing no serum, is control against spontaneous agglutination.

If the limit of agglutination is not reached within this series, higher dilutions are followed out in a similar manner.

Thus, for example, 57 drops of normal saline solution plus 3 drops of a 1 in 10 serum dilution will give a serum dilution of 1 in 200, and, using the same quantities as before, one has the serum acting in dilutions of 1 in 500, 1 in 1000, 1 in 2500, and 1 in 5000. And similarly for higher dilution.

(h) The tubes are examined after two hours at 50° to 55° C., followed by fifteen minutes' standing at room temperature. The reading is taken by comparing each tube in succession with the control tube, and is preferably made by means of artificial light against a black background. If daylight is used, the tubes inspected should be partly shadowed by passing a finger up and down behind them.

A reading-glass aids in making finer and more delicate readings.

If time permits it is preferable to place the tubes in a refrigerator over night making the readings next morning.

Dreyer and Inman¹ have described standard agglutination, standard agglutinin unit (the unit of agglutinating power), the reduction factor and method of readings as follows:

Standard agglutination is the degree of agglutination present in the highest serum dilution in which marked agglutination without sedimentation can be seen by the naked eye.

The *standard agglutinin unit* is that amount of agglutinating serum which when made up to 1 c.c. volume with normal saline solution causes standard agglutination on being mixed with 1.5 c.c. of the original standard agglutinable culture and maintained at 55° C. for two hours (in the case of dysentery agglutination four and a half hours) in a water-bath, followed by fifteen to twenty minutes at the room temperature.

The Reduction Factor.—The total volume in which the reaction occurs being 2.5 c.c. (1 c.c. of serum added to 1.5 c.c. of standard culture) the original standard agglutinable culture was given the reduction factor of 2.5 to express the sensitiveness to agglutination of that particular culture. All subsequent batches of culture have been given reduction factors calculated on this basis, thus securing constancy in the agglutinin unit. For example, if a batch of standard culture proves to be twice as sensitive to agglutination as the original standard, so that half the amount of serum produces standard agglutination under test conditions, the new standard culture is given a reduction factor of double the size of the original factor, *i. e.*, 5. Thus, whatever be the particular standard culture used to test any given serum the number of agglutinin units found per cubic centimeter of the serum remains always the same, although the dilutions in which standard agglutination occurs will be different. Since when standard agglutination occurs in a serum dilution of 1 in x , then x divided by the reduction factor for the particular standard agglutinable culture used gives the number of standard agglutinin units contained in 1 c.c. of the serum concerned.

Readings.—Owing to the rate at which the dilution increases in the series of tubes employed it will commonly happen that no tube in the series exhibits standard agglutination. If this be so, it will be found in looking

¹ The Lancet, London, March 10, 1917.

along the series that while one tube shows strong agglutination with sedimentation the next succeeding tube shows no agglutination or only a trace. In such cases standard agglutination lies approximately midway between the two dilutions. Though this method of making readings is amply adequate for diagnostic purposes it will be found that should a more precise determination of the limits of agglutination be required it can be obtained by using a stand of 12 tubes with the series of quantities given in the table contained in the directions for preparation and standardization of agglutinable cultures, where the successive dilutions of the serum only differ by about 20 per cent. Almost as accurate a reading can, however, be obtained with experience from the short series by taking note of the *degree of agglutination* present in each tube and by using a suitable interpolation table.

The principal terms employed in describing the different degrees of agglutination met with are "total" (*t*), "standard" (*S*), "trace" (*tr*), and "mil." (*0*). *Total* agglutination indicates the condition in which the whole, or practically the whole, of the agglutinated bacteria have settled down at the foot of the tube. *Standard* agglutination has already been described; the term *Trace* is applied to a very fine granulation recognizable by the naked eye. Around these main terms subsidiary differences congregate themselves as follows: *Total minus* (*t* -) marked deposit, but a number of floating flocculi remaining in the fluid. On each side of "standard" we find *Standard plus* (*S* +), and *Standard minus* (*S* -) respectively. In the former no deposit, but much larger flocculi than are seen in standard agglutination. In the latter finer agglutination than standard, with more the appearance of granulation in the fluid. Similarly, we recognize a *Trace plus* (*tr* +), and a *Trace minus* (*tr* -), the former representing something more than *trace*, but less than *standard minus*, the latter being on the limit of naked-eye visibility. Finally, on occasion it can be difficult to decide with certainty whether a given tube is absolutely nil or not, the term *query trace* (*tr* ?) is then applied.

Modified Dreyer Test.—A drawback to the accuracy of Dreyer's technic consists in the measurement of fluids by drops; Donald¹ has emphasized the importance of this source of error, although Walker² has replied to the criticisms and maintains that the drop method is accurate.

I have found the use of accurately graduated 1 c.c. pipets preferable for measuring all fluids; likewise the use of larger amounts of serum dilutions and bacterial emulsion, the tests being set up as follows:

Serum is diluted 1 in 10 by mixing 0.2 c.c. in a test-tube with 1.8 c.c. distilled water or physiologic saline solution.

Tube 1	— 1.0 c.c. of serum	1 : 10 + 1.5 c.c. culture	= 1 : 25.
Tube 2	— 0.5 c.c. of serum	1 : 10 + 1.5 c.c. culture	= 1 : 50.
Tube 3	— 0.2 c.c. of serum	1 : 10 + 1.5 c.c. culture	= 1 : 125.
Tube 4	— 0.1 c.c. of serum	1 : 10 + 1.5 c.c. culture	= 1 : 250.
Tube 5	— 1.0 c.c. of water	+ 1.5 c.c. culture	= control.

These give the same final dilutions as employed by Dreyer. The balance of the test is exactly as described above.

Macroscopic Slide Methods.—For the rapid identification of bacteria in bacteriologic studies Coca³ has described an efficient method for conducting agglutination tests on slides; Krumwiede⁴ has found the method

¹ The Lancet, London, September 2, 1916, 423.

² The Lancet, London, September 23, 1916.

³ Bull. Manila Med. Soc., 1910, 2, No. 1.

⁴ Jour. Infect. Dis., 1918, 23, 275.

satisfactory for the identification of typhoid, paratyphoid, and dysentery bacilli isolated from feces and meningococci from the nasopharynx. He has also described a dropping bottle for use in these tests.¹ The technic is as follows:

1. The tests are conducted with highly potent immune serum. The dilutions may be as low as 1 : 50 or 1 : 100. With a known and tested serum used in proper dilution the results are very reliable; with an unknown serum serious error may result because of group reaction (Krumwiede). Occasionally a slight delay in clumping due to the proagglutination phenomenon will be noticed, but there is usually some evidences of agglutination.

2. A drop of diluted immune serum is placed on a slide; a drop of saline solution is placed on the same or a second slide, due care being taken to avoid admixture with serum.

3. A portion of the suspicious colony is picked off with a small loop of fine wire, and a sufficient amount rubbed off in the salt solution to give a slight clouding (this is the control). Some of the growth remaining on the loop is then rubbed off similarly in the drop of diluted serum.

4. In a positive reaction clumping occurs almost immediately; spontaneous agglutination is detected in the control.

Bass and Watkins² have described a slide method for conducting the Widal test for typhoid fever which is very simple and may be conducted by the physician at the bedside or in his office. The technic is as follows:

1. The culture is prepared by suspending twenty-four-hour growths of typhoid bacilli in distilled water, 10,000,000,000 per cubic centimeter, killed and preserved with 1 per cent. commercial formalin. This suspension is said to keep six to twelve months or longer. It must be well shaken before using.

2. A blood film is made on a slide in the usual manner, using approximately $\frac{1}{4}$ drop of blood.

3. Place on the blood 1 drop of water and dissolve the blood with the aid of a tooth-pick or other suitable instrument.

4. Add 1 drop of the suspension of typhoid bacilli, and mix by tilting the slide from side to side and from end to end, causing the mixture to flow back and forth.

5. A positive reaction occurs within two minutes, with the formation of small grayish clumps and fine granular sediment. When the test is negative no such granular sediment forms. Dust particles must not be mistaken for agglutinated bacilli.

Technic of Conglutination Test with Bacteria.—Fresh bovine serum is heated to 56° C. for one-half hour and tested for agglutinin for the bacteria under study. If agglutinin is present it is removed by adding to each 5 c.c. of serum about 10 loopfuls of the corresponding bacteria, mixing well, and removing the clumps by thorough centrifuging and filtration through paper after standing at room temperature for several hours. It may be necessary to repeat this step once or twice more.

Dilutions of the patient's serum in amounts of 1 c.c. are made in small clean test-tubes, as in the macroscopic agglutination test. To each tube add 0.1 c.c. of bovine serum ("conglutinin"); 0.1 c.c. of fresh guinea-pig serum (complement), and 0.1 c.c. of the emulsion of the bacteria of sufficient density to give well-marked emulsions in the test-tubes. Controls of bovine serum, complement serum, and patient's serum should be included; also a culture control prepared with normal salt solution. After gentle mixing and standing at room temperature for twenty-four hours the results are

¹ Jour. Immunology, 1920, 5, 155.

² Archiv. Int. Med., 1911, 8.

read; positive results are indicated by complete clearing of the tube with the micro-organisms in flakes or granules, either clinging to the sides of the tube as granular material or at the bottom as flaky granular sediment.

The Agglutination Test in the Differentiation of Pneumococci.—The investigations of Neufeld and Händel, and particularly of Cole, Dochez, and their associates in the Rockefeller Institute, have resulted in the grouping of pneumococci into four groups on the basis of agglutination and protective tests. Group III is composed of pneumococci belonging to the type of *Pneumococcus mucosus*, and an efficient antiserum has not yet been produced; Group IV is composed of all pneumococci not falling into the first three groups.

The agglutination test is conducted for the purpose of differentiating these types; if it is decided to administer an immune serum, the serum corresponding to the type of infection must be given. The following method has been described by Avery, Chickering, Cole, and Dochez¹:

Collection of Sputum.—Care should be exercised in the collection of sputum to obtain a specimen from the deeper air-passages as free as possible from saliva. This can be done in practically all cases, even the most difficult, with a little persistence. The sputum is collected in a sterile Esmarch dish or other suitable container, and should be sent at once to the laboratory for examination. When delay is unavoidable the specimen should be kept on ice during the interval.

Microscopic Examination of Sputum.—Direct films of sputum are stained by Gram, Ziehl-Neelsen, and by Hiss capsule stains. This serves to give an idea of the nature of the organisms present and an indication of the source of the sputum. Suitable lung specimens of sputum are relatively free in most instances from contaminating mouth organisms. It is frequently possible to identify Type III (*Pneumococcus mucosus*) organisms when they are present, as they possess very large distinct capsules staining by both Gram's and Hiss' methods.

Mouse Inoculation.—A small portion of the sputum about the size of a bean is selected and washed through three or four changes of sterile salt solution in sterile Esmarch or Petri dishes to remove surface contaminations. When the sputum is too friable or when the specimen is relatively free from secondary organisms, this washing process may be omitted. In either event the kernel of sputum selected is transferred to a sterile mortar, ground up, and emulsified with about 1 c.c. of sterile bouillon or salt solution, added drop by drop, until a homogeneous emulsion is obtained that will readily pass through the needle of a small syringe. With a sterile syringe 0.5 to 1 c.c. of this emulsion is inoculated intraperitoneally into a white mouse. The pneumococcus grows rapidly in the mouse peritoneum, while the majority of other organisms rapidly die off with the exception of Friedländer's bacillus, *Bacillus influenzae*, and occasionally *Micrococcus catarhalis*, staphylococcus, and streptococcus. Pneumococcal invasion of the blood-stream also occurs early. *Bacillus influenzae*, if present, likewise invades the blood-stream; other organisms, as a rule, do not. The time elapsing before there is sufficient growth of pneumococcus in the mouse peritoneum for the satisfactory determination of type varies with the individual case, depending upon the abundance of pneumococci in the specimen of sputum and the virulence and invasiveness of the strain present. It may be from five to twenty-four hours, averaging six to eight hours with the parasitic fixed Types Nos. I, II, and III. As soon as the injected mouse appears sick, a drop of peritoneal exudate is removed by means of peritoneal

¹ Monograph No. 7, Rockefeller Institute, 1917, 23.

puncture with a sterile capillary pipet, spread on a slide, stained by Gram's method, and examined microscopically to determine whether there is an abundant growth of pneumococcus present. If there is an abundant growth of pneumococcus alone the mouse is killed and the determination of type proceeded with. If the growth is only moderate, or if other organisms are present in any quantity, further time must be allowed until subsequent examination of the peritoneal exudate shows an abundant growth of pneumococcus. It should be emphasized that undue haste in killing the mouse is time lost in the end.

Mouse Autopsy.—As soon as the mouse is killed or dies, the peritoneal cavity is opened with sterile precautions and cultures are made from the exudate in plain broth and on one-half of a blood-agar plate. Films are made and stained for microscopic examination by Gram's stain and Hiss' capsule stain. The peritoneal exudate is then washed out by means of a sterile glass pipet with 4 to 5 c.c. of sterile salt solution, the washings being placed in a centrifuge tube. Cultures are then made from the heart's blood in plain broth and on the other half of the blood-agar plate.

Agglutination Test.—When the pneumococcus is present in pure culture in the peritoneal exudate, the determination of type may satisfactorily be made by macroscopic agglutination tests as follows: (1) The peritoneal washings are centrifuged at low speed for a few minutes until the cells and fibrin contained in the exudate are thrown down. The supernatant bacterial suspension is transferred into a second centrifuge tube and centrifuged at high speed until the organisms are thrown out. The supernatant fluid is saved for precipitin tests (see chapter on Precipitins), and the bacterial sediment taken up in sufficient salt solution to make a moderately heavy suspension. The concentration of bacteria should be similar to that of a good eighteen-hour broth culture of pneumococcus.

(2) Highly potent antipneumococcus sera for Types I, II, and III must be available, at least for Types I and II; if these sera are cloudy they should be centrifuged before use.

(3) The test is set up as follows in 6 small test-tubes:

Tube 1: 0.5 c.c. Serum I (1 : 10) + 0.5 c.c. bacterial suspension = 1 : 20.

Tube 2: 0.5 c.c. Serum II (undiluted) + 0.5 c.c. bacterial suspension = 1 : 2.

Tube 3: 0.5 c.c. Serum II (1 : 10) + 0.5 c.c. bacterial suspension = 1 : 20.

Tube 4: 0.5 c.c. Serum III (1 : 5) + 0.5 c.c. bacterial suspension = 1 : 10.

Tube 5: 0.1 c.c. Sterile ox bile + 0.5 c.c. bacterial suspension.

Tube 6: 0.5 c.c. saline solution + 0.5 c.c. bacterial solution (control) suspension.

Tube 5, containing bile plus bacterial suspension, is to determine the bile solubility of the strain and for differentiation of pneumococcus from streptococcus; pneumococcus is bile soluble and the contents become clear.

Tube 6 is the control for spontaneous agglutination.

(4) The tubes are gently shaken and incubated in a water-bath for one hour at 37° C., when the readings are made.

(5) Positive reactions are usually readily detected and may be brought out clearly by gentle agitation of the tubes. If no agglutination has occurred and if the bacteria are bile soluble and otherwise resemble the pneumococcus, the type is IV. If agglutination occurs in tubes 2 and 3 there is present typical Type II pneumococci; if agglutination is partial in tube 2 and absent in tube 3 atypical Type II pneumococci are present. If agglutination occurs only in tube 1 the pneumococcus is Type I; if only in tube 4 it belongs to Type III. Sometimes two types occur together and Type IV

may occur with any of the other types without being detected by this test. Sometimes the readings are facilitated by standing the tubes aside for several hours before making the readings.

The test may be conducted with *young* white rats inoculated with two to four times the amount of sputum recommended for mice; the latter, however, are more satisfactory.

Avery¹ has recommended a method for conducting the test when animals are not available, consisting of inoculating the prepared sputum in special dextrose broth medium for securing the initial growth of pneumococcus for the tests; it is not as efficient as the mouse test described above.

Microscopic tests are conducted by mixing on cover-slides platinum loopfuls of bacterial emulsion and undiluted and diluted immune sera. These slides are then suspended as hanging-drop preparations and the results read after one-half to an hour. A control should always be included. Results are not quite as accurate as those secured by the macroscopic test.

The Agglutination Test in the Differentiation of Meningococci.—The specific agglutinins of the meningococcus have been the subject of much investigation. As a result it has been shown that differences exist, and from a study of these variations and the application of "absorption" methods, it has been possible to differentiate the majority of strains into groups or types. Gordon recognizes four types; according to Flexner, Gordon's Type I appears to correspond with the "parameningococcus" of Dopter, and Type II with the normal or regular meningococcus. Types III and IV appear to conform to the more common intermediates.

Type Sera.—These may be prepared by the immunization of horses, but for small amounts young rabbits are generally employed. Gordon and Murray inject 0.5 c.c. of a suspension (1,000,000,000 cocci) intravenously, and forty-eight hours later give three additional doses of 0.5 c.c. at hourly intervals. By this method within ten days a serum may be obtained with a titer as high as 1 : 800. Hine secured better results by injecting 1,000,000,000 cocci as an initial dose followed by 500,000,000 one hour later, and six days later by 3,000,000,000. The serum is tested on the eighth day.

Suspension.—A suspension of the coccus is made in sterile saline and heated for half an hour at 65° C. to kill the cocci and inactivate the autolysins. The suspension is standardized so that each cubic centimeter contains approximately 2,000,000,000 cocci, and 0.5 per cent. phenol added. This phenolated suspension may be used for agglutination tests and for the immunization of rabbits. Agglutination tests may be conducted with the cloudy spinal fluids of persons ill with meningococcus meningitis in which smears show large numbers of extracellular cocci after the fluid has been briefly centrifuged to remove pus-cells.

Test.—Four dilutions of each serum are prepared with saline solution in small tubes in amounts of 1 c.c., 1 : 25, 1 : 50, 1 : 100, 1 : 200. To each tube and a control carrying 1 c.c. of saline solution is added 1 c.c. of bacterial suspension. Results are read after sixteen hours' incubation at 55° C.

For the "absorption" of group agglutinins the sera are saturated with a suspension of the coccus to be tested for twenty-four hours, after which they are centrifugalized. The supernatant clear sera are then put up against their homologous cocci.

Gates² has recently described a centrifuge method for the serologic grouping of meningococci. Ellis³ and Gates⁴ have also described simple macroscopic methods in capillary tubes.

¹ Jour. Amer. Med. Assoc., 1918, 70, 17.

³ Brit. Med. Jour., 1915, 2, 881.

² Jour. Exper. Med., 1922, 35, 63.

⁴ Jour. Amer. Med. Assoc., 1921, 77, 2054.

TECHNIC OF THE ABSORPTION AGGLUTINATION TEST IN MIXED INFECTION (THE SATURATION TEST OF CASTELLANI)

The practical importance of partial agglutinins is recognized in the diagnosis of mixed infections. Thus the serum of a patient may agglutinate typhoid as well as paratyphoid bacilli in dilutions up to 1 : 100. This may indicate one of three possibilities:

1. The patient may be infected with typhoid, but has formed an exceptionally large quantity of group agglutinins for paratyphoid bacilli. Saturation of this serum with typhoid bacilli will remove all the typhoid and a portion, if not all, of the group agglutinins. Saturation with paratyphoid bacilli will remove the group agglutinins, but not the main or typhoid agglutinin.

2. The patient may be infected with paratyphoid bacilli, but has formed, at the same time, many partial agglutinins for typhoid bacilli. Saturation of the serum with paratyphoid bacilli will remove all the paratyphoid and a large portion of the typhoid agglutinin.

3. The patient may have a mixed infection of typhoid and paratyphoid, and therefore agglutinin for both may be present. Saturation of the serum with typhoid bacilli will remove the typhoid and probably a small portion of the paratyphoid agglutinin. After this reaction the serum will still show the presence of a decided quantity of paratyphoid agglutinin.

In selecting the most likely one of these hypotheses a decision may be reached by adopting the method of Castellani (Citron), which is as follows:

1. Four rows of test-tubes are arranged, each row being made up of four small tubes each containing 1 c.c. of serum dilutions 1 : 20, 1 : 40, 1 : 80, and 1 : 160, respectively.

2. In each of the tubes of the first and second rows five loopfuls of typhoid bacilli are emulsified. An extra tube containing 1 c.c. of normal salt solution receives a similar amount of bacteria, and serves as the typhoid control.

3. In each tube of the third and fourth rows five loopfuls of paratyphoid bacilli are emulsified. Arrange the paratyphoid culture control.

4. Mix gently and incubate for four hours. Carefully record the presence or absence of agglutination in each test-tube. Centrifuge all the tubes excepting the two controls, and transfer the supernatant fluid of each to other test-tubes arranged in the same order.

5. To each tube of the first and third rows add five loopfuls of typhoid bacilli; to each of the second and fourth rows, five loopfuls of paratyphoid bacilli. Mix well and incubate for four hours.

(a) If typhoid is present, the agglutination titer in the first part of the test will be strong in the tubes of the first and second rows, and weak in those of the third and fourth rows. In the second part of the test the titer for typhoid will be weak or nil in the first, second, and fourth rows, whereas in the third row it will remain practically the same.

(b) If paratyphoid exists, the agglutination titer in the first part of the test will be strong in the tubes of the third and fourth rows, and weak in those of the first and second rows. In the second part of the test the titer for paratyphoid will be less or negative in the fourth row, and strong or unchanged in the second row.

(c) If a mixed infection exists, the agglutination titer in the first part of the test will be strong in the tubes of all four rows. In the second part of the test the titer in the first and fourth rows is much weaker or negative, and in the second and third rows it will remain the same.

Technic of Acid Agglutination.—Mixtures of lactic acid and sodium

lactate have been generally employed. Gillespie has prepared these from stock solutions of one-third normal sodium lactate, with a small crystal of thymol, and normal lactic acid, according to the following scheme:

$\frac{N}{3}$ sodium lactate in c.c.	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Normal lactic acid in c.c.	0.12 ¹	0.25	0.5	1.0	2.0	0.5	1.0	2.0	4.0	8.0	16.0
Distilled water in c.c. . .	8	8	8	8	8	18.0	17.5	16.5	14.5	10.5	2.5
Ratio of acid to salt . . .	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	1	2	4	8	16	32
H-ion concentration in grams per multiplied by 10 ⁴	0.04	0.1	0.2	0.4	0.7	1.4	2.8	5.5	11	22	44

A series of greater dilutions of lactates may be made by diluting each of the above with one or more volumes of distilled water.

Young cultures of bacteria should be employed; if grown in broth, they may be prepared by rapid centrifuging and resuspension in sufficient distilled water to secure an even emulsion of such density as suitable for the macroscopic agglutination test—0.3 c.c. of the bacterial emulsion is placed in each of a series of small and perfectly clean tubes in a rack, and 1 c.c. of the proper reaction mixture added to each tube. The tubes are then gently and briefly shaken, and placed in a water-bath at about 37.5° C. for an hour or two. Michalis² uses acetic acid and reports sharp differentiation of *Bacillus typhosus* by the method of acid agglutination.

¹ Read 0.12 c.c. of normal acid freshly diluted 1 : 8.

² Deutsch. med. Wchn., 1915, xli, 241.

CHAPTER XVI

HEMAGGLUTININS

As previously stated, agglutination like other immunity reactions is a manifestation of broad biologic laws and is not limited to bacteria. Agglutinins for other cells including erythrocytes, leukocytes, epithelium, and spermatozoa may be found in the sera of some animals either normally or naturally or as a result of immunization. From the standpoint of practical importance the hemagglutinins in human serum for human corpuscles (iso-agglutinins) are of most interest and especially in relation to the transfusion of blood.

Substances Causing Hemagglutination.—The agglutination of erythrocytes may be caused by many different non-specific and specific agents which may be classified as follows:

(a) *Various inorganic colloids* may cause the agglutination of thin suspensions of blood corpuscles as shown by Landsteiner and Jagic,¹ Hirschfeld,² and others with solutions of silicic acid and other substances.

(b) *Various plant substances* or *phyto-agglutinins*, as abrin, ricin, and crotin. Abrin and ricin agglutinate the corpuscles of practically all warm- and cold-blooded animals; crotin agglutinates the corpuscles of the sheep, swine, horse, and to some extent the rabbit, but not of the dog. The seeds of many non-poisonous leguminous plants, and also of *Solanaceæ*, yield extracts that are strongly agglutinative for red corpuscles, the active substances being usually found in the proteose fraction.

(c) *Bacterial substances* may cause hemagglutination as first shown by Kraus and Ludwig³ with bouillon cultures of staphylococci and various other bacteria. These have been studied extensively by Pearce and Winnie,⁴ and the phenomenon is sometimes seen in tests for bacterial hemolysins.

(d) *Animal secretions*, as snake venoms, contain agglutinins for the corpuscles of the rabbit, guinea-pig, dog, sheep, and other animals as discovered by Mitchell and Stewart,⁵ and Flexner and Noguchi.⁶ These agglutinins are usually destroyed by heating to 75° C., and their agglutinating activity is usually in inverse ratio to their hemolytic activity.

Various tissue extracts may contain hemagglutinins as shown by Sick⁷ with extracts of organs of normal cats and dogs. Apparently these substances are derived from the cells, as the blood-serum is free of agglutinins. Romer⁸ has observed agglutinins for rabbit corpuscles in extracts of the lens of the rabbit, and Landsteiner⁹ observed agglutinins in saline extracts of malignant tumors.

Hemagglutinins have also been found in milk and colostrum by Landsteiner,¹⁰ Langer,¹¹ Kraus,¹² and others; in urine by Pfeiffer,¹³ Friedberger,¹⁴

¹ Wien. klin. Wchn., 1904, No. 3; Münch. med. Wchn., 1904, No. 27.

² Arch. f. Hyg., 1907, 63, 237.

³ Wien. klin. Wchn., 1902, 59.

⁴ Amer. Jour. Med. Sci., 1904, 128, 669.

⁵ Trans. College of Phys. of Phila., 1897, 19, 105.

⁶ Jour. Exper. Med., 1902, 6, 277.

⁷ Deutsch. Arch. f. klin. Med., 1904, 138, 389.

⁸ Arch. of Ophthal., 1905, 60, 239.

⁹ Wien. klin. Wchn., 1908, No. 45.

¹⁰ Münch. med. Wchn., 1903, 1812.

¹¹ Ztschr. f. Heilk., 1903, 24, 111.

¹² Wien. klin. Wchn., 1901, 737.

¹³ Ztschr. f. Hyg., 1907, 56, 488.

¹⁴ Berl. klin. Wchn., 1900, 1236.

and others, and in cyst fluids by Brinkerhoff and Southard.¹ They are only occasionally found in spinal fluid.

Tallqvist² has found hemagglutinins in extracts of *Bothriocephalus*.

(e) *Serum hemagglutinins* are of most importance, and have received most attention, and especially in relation to the subject of blood transfusion.

Kinds of Serum Hemagglutinins.—1. *Normal or natural hemagglutinins* or those found in normal sera. These are of three kinds:

(a) Autohemagglutinins, those that agglutinate the corpuscles of the same animal.

(b) Isohemagglutinins, those that agglutinate the corpuscles of another animal of the same species.

(c) Heterologous hemagglutinins, those that agglutinate the corpuscles of animals of different species.

2. *Immune hemagglutinins*, those produced by immunizing an animal with injections of blood from an animal of different species. A familiar example of these are the agglutinins for human erythrocytes to be found in the sera of rabbits immunized with human blood-corpuscles in the production of antihuman hemolysin.

AUTOHEMAGGLUTININS

Auto-agglutination, that is, agglutination of red blood-corpuscles by the serum of the same individual, is an extremely rare phenomenon. In 1902 Klein³ observed auto-agglutination in the blood of a horse and possibly also in the blood of a human being suffering with cirrhosis of the liver. Landsteiner⁴ noted auto-agglutinins in the sera of horses and other animals. Hektoen⁵ has reported the occurrence of these agglutinins in the blood of two individuals, and Ottenberg and Thalhimer⁶ in the blood of cats.

Rous and Robertson⁷ have made the interesting observation that repeated blood transfusions of rabbits with rabbit blood leads to the production of auto-agglutinins; also that repeated bleedings lead to the same results.⁸ Both observations are of considerable interest in connection with blood transfusion.

Recently Clough and Richter⁹ have reported and studied auto-agglutination with the serum of a man suffering with bronchopneumonia. They found that this auto-agglutinin differed from ordinary agglutinins in various ways as follows: (1) It was active only at low temperatures, the agglutination breaking up on warming confirming Landsteiner's observations in this regard. (2) It was absorbed from the serum only at low temperature, and was liberated from the cells on warming. (3) It was active on red blood-corpuscles of other animals. Absorption tests showed that this auto-agglutinin was distinct from other hetero-agglutinins and iso-agglutinins for corpuscles of Groups 1 and 2 contained in the serum of this individual. Auto-agglutinin was also found in the serum of a daughter of the patient, indicating a hereditary peculiarity. Kligler¹⁰ has also described autohemagglutinins in the serum of a pregnant woman suffering from a chronic heart

¹ Jour. Med. Research, 1903, 9, 28.

² Ztschr. f. klin. Med., 1907, 61, 427.

³ Wien. klin. Wchn., 1902, 15, 413.

⁴ Münch. med. Wchn., 1903, 40, 1812; *ibid.*, 1902, 39, 1905.

⁵ Jour. Infect. Dis., 1907, 4, 297.

⁶ Jour. Med. Res., 1915, 33, 213.

⁷ Jour. Exper. Med., 1918, 27, 509.

⁸ Jour. Exper. Med., 1918, 27, 563.

⁹ Johns Hopkins Hosp. Bull., 1918, 29, 86.

¹⁰ Jour. Amer. Med. Assoc., 1922, 78, 1195.

lesion, and in a condition of severe anemia due to repeated hemorrhages from hemorrhoids. Auto-agglutination occurred only at room temperature and not at 37° C.; furthermore, agglutinated masses of corpuscles at room temperature were dispersed when warmed.

ISOHEMAGGLUTININS

Agglutinins in human sera for the corpuscles of other human beings have assumed considerable practical importance in relation to blood transfusion and particularly of adults.

Transmission and Influence of Age.—Halban¹ was first to note that agglutinins may be absent from the blood of an infant while present in that of the mother. Unger² found agglutinins in only 13 per cent. of infants under one month of age, although present in 97 per cent. of adults. The percentage of children whose sera contained agglutinins increased with age, but reached the adult average only between the second and fourth years of life. Likewise, the corpuscles of infants cannot be agglutinated by any serum in the majority of instances. According to Happ³ and Hess⁴ the cells of newborn infants are rarely agglutinated, and the grouping present in adults rarely present in blood from the umbilical vein. The acquisition of susceptibility to agglutination occurs at about six months and far earlier than the appearance of agglutinins in the serum. At about two years of age all children have probably established their adult iso-agglutinin group (Happ). For these reasons Unger believes it is safe to transfuse an infant of six months or less with the blood of any group, but Happ states that the agglutinins in the blood of mother and child may be different and that it is not safe to transfuse an infant from its mother without making preliminary tests. Furthermore, Jones (A.) has recently reported that 78.7 per cent. of 197 specimens of infant blood examined could definitely be placed in one of the four recognized groups. These results seemed to be dependent on a technic which permitted the recognition of weak agglutinins. Isohemolysins were also found in 13.7 per cent. of sera, and because of these iso-agglutinins and isohemolysins Jones also advises that compatibility blood tests be made in selecting a donor for transfusion of infants.

Of interest in this connection it may be stated that Happ found agglutinins in the milk of 14 nursing women identical in grouping to those found in their sera. Of the 14 infants only 5 showed the presence of agglutinins, and Happ concludes that the infant does not acquire agglutinins through the mother's milk.

According to H. and L. Hirschfeld,⁵ Verzar and Weszecszy,⁶ and Lewis and Henderson⁷ the distribution of four groups of hemagglutinins varies extremely, but certain races could be grouped corresponding somewhat to the grouping made on an anthropologic basis and still more completely according to geographic distribution of the races.

Classification and Grouping.—Landsteiner⁸ is credited with having discovered that human sera contain more than one iso-agglutinin; this discovery has been amply confirmed and has led to various classifications of the iso-agglutinins by different investigators and much confusion.

¹ Wien. klin. Wchn., 1900, 13, 545; Münch. med. Wchn., 1902, 49, 473.

² Jour. Amer. Med. Assoc., 1921, 76, 9.

³ Jour. Exper. Med., 1920, 31, 313.

⁴ Deut. med. Wchn., 1921, 47, 241.

⁵ Lancet, 1919, 2, 675.

⁶ Biochem. Ztschr., 1921, 126, 33.

⁷ Jour. Amer. Med. Assoc., 1922, 79, 1422.

⁸ Centralb. f. Bakteriöl., Abt., 1900, 27, 357.

Landsteiner expressed the belief that human sera contain two iso-agglutinins and that the corpuscles may be divided into two kinds according to their susceptibility to agglutinins and two agglutinogens. This work was quickly confirmed by the investigations of Shattock,¹ Donath,² Grunbaum,³ von Descatello and Sturli,⁴ Eisenberg,⁵ and others who studied principally the bloods of sick persons, with the result that the presence of these iso-agglutinins were considered pathologic until Landsteiner⁶ showed that they occurred in normal blood and could be divided into three main groups. von Descatello and Sturli added a fourth group, and Hektoen⁷ also found three iso-agglutinins in human sera instead of the two described by Landsteiner.

The next important contributions were made by Jansky⁸ and Moss,⁹ who divided the blood of all human beings into four groups. *At the present time the classification of Landsteiner plus the fourth group described by von Descatello and Sturli, the classification of Jansky and that of Moss, are usually described in text-books, and inasmuch as they do not correspond has led to a great deal of confusion, and the possibility of error and danger in blood transfusion.*

Jansky's classification is largely employed in Europe and that of Moss in America; in order to standardize the practice of grouping blood and remove the danger of using both systems, a committee composed of members of the American Association of Immunologists, the Society of American Bacteriologists, and the Association of Pathologists and Bacteriologists have recently reviewed the subject, pointed out the dangers, and recommended the adoption of one classification in order to avoid confusion and the possibility of accident; on the basis of priority the classification of Jansky was recommended.¹⁰

The following table shows how the groups of Jansky correspond to those of Landsteiner and Moss:

RELATION OF DIFFERENT CLASSIFICATIONS OF HUMAN ISO-AGGLUTININS

Jansky's.	Corresponds to:	
	Landsteiner's.	Moss'.
Group I	Group C	Group IV
Group II	Group A	Group II
Group III	Group B	Group III
Group IV	Descatello-Sturli Group	Group I

From this table it will be noted that the classifications of Jansky and Moss are identical excepting that Groups I and IV are interchanged.

Karsner¹¹ has recently summarized the average incidence of these groups

¹ Jour. Path. and Bacteriol., 1900, 6, 303.

² Wien. klin. Wchn., 1900, 13, 497.

³ Brit. Med. Jour., 1900, 1, 1089.

⁴ Münch. med. Wchn., 1902, xlix, 1090.

⁵ Wien. klin. Wchn., 1901, 14, 1020.

⁶ Wien. klin. Wchn., 1901, 14, 1132.

⁷ Jour. Infect. Dis., 1907, 4, 297.

⁸ Sborn. Klin., 1907, 8, 85.

⁹ Bull. Johns Hopkins Hosp., 1910, 21, 63.

¹⁰ Jour. Amer. Med. Assoc., 1921, 76, 130.

¹¹ Jour. Amer. Med. Assoc., 1921, 76, 88.

based upon more than 5000 tests made by five different investigators on the basis of Jansky's classification; I have modified his table to show its relation to the Moss classification:

INCIDENCE OF THE FOUR GROUPS OF ISO-HEMAGGLUTININS IN MAN

Jansky.	Corresponds to Moss.	Incidence.
Group I	Group IV	42.84 per cent.
Group II	Group II	41.38 per cent.
Group III	Group III	10.36 per cent.
Group IV	Group I	5.42 per cent.

It will be noted that according to the Jansky classification Groups I and II (corresponding to Moss' Groups II and IV) preponderate, and collectively constitute about four-fifths of the race; the percentages observed by Culpepper and Ableson¹ on the basis of 5000 tests were similar.

The technic of grouping is described later in this chapter; the important relation of grouping to blood transfusion and the reactions following this operation are discussed under Blood Transfusion.

Subgroups.—The possible existence of specific subgroups or minor agglutinins is still an open question. Langer² claimed that one serum he examined contained six iso-agglutinins demonstrable by successive absorptions with different agglutinable corpuscles. Culpepper and Ableson³ and Unger⁴ believe that the groups may "overlap" and thus explain reactions between members of the same group. For this reason Unger advises that recipient and donor be tested directly against each other, and that it should not be assumed that bloods are compatible merely because typing with Groups II and III sera show that they belong in the same group.

Isohemagglutinins in the Lower Animals.—Hektoen was not able to find iso-agglutinins in the serum of rabbits, guinea-pigs, dogs, horses, and cattle; as far as I am aware these observations have not been disputed. The subject is worthy of further investigation by reason of the unique situation as compared with human sera, and especially so since heterologous hemagglutinins occur in the sera of the lower animals.

Heterologous Hemagglutinins in Human Blood.—As previously stated, these are agglutinins in human blood for the corpuscles of the lower animals. They have been studied largely in relation to the Wassermann test as influencing the choice of corpuscles for the indicator antigen. In studies of this kind Kolmer, Matsunami, and Trist⁵ found agglutinins in the unheated sera of human beings for the corpuscles of a number of the lower animals in the following percentages:

For sheep corpuscles.....	16 per cent.
For chicken corpuscles.....	10 "
For guinea-pig corpuscles.....	28 "
For ox corpuscles.....	occasionally

¹ Jour. Lab. and Clin. Med., 1921, 6, 276.

² Ztschr. f. Heilk., Abt. Int. Med., 1903, 24, 111.

³ Loc. cit.

⁴ Jour. Amer. Med. Assoc., 1921, 76, 9.

⁵ Amer. Jour. Syph., 1919, 3, 407.

IMMUNE HEMAGGLUTININS

These agglutinins are prepared by injecting the corpuscles of one animal into a second animal of a different species. Curiously the injection of rabbits with human corpuscles is followed by the production of agglutinins to a somewhat greater degree than occurs when rabbits are injected with sheep corpuscles or those of the ox. They are best produced by injecting washed corpuscles, but are also produced by injecting whole blood and even serum. Their presence in immune serum is disturbing in complement-fixation tests, and particularly antihuman agglutinin in rabbit antihuman hemolytic serum. So far no adequate method has been described for the complete removal of these agglutinins without removal of the hemolysins at the same time; desiccation, however, tends to destroy the agglutinins somewhat more than the hemolysins as shown by the studies of Sands and West¹ in my laboratory.

The immune hemagglutinins were apparently first observed by Belfanti and Carbone in the serum of a horse injected with rabbit blood. As shown later by Landsteiner² and von Dungern³ heating immune serum for thirty minutes to 55° to 60° C. may remove hemolysins, but not hemagglutinins.

Curiously the injection of rabbits with goose blood may produce agglutinins not only for goose corpuscles, but raise considerably the amount of normal or natural agglutinins in rabbit serum for the corpuscles of the guinea-pig, sheep, and other animals. A similar result is observed when rabbits are injected with guinea-pig corpuscles.

Immune Isohemagglutinins.—Human sera must be used for the typing of human corpuscles. Since these do not keep well Kolmer and Trist⁴ attempted to prepare immune hemagglutinins for the four groups of human corpuscles by injecting rabbits with cells of the four different groups. Our antisera often showed somewhat higher titers for cells of the group used in immunization, but absorption to remove group antibodies resulted in complete exhaustion of agglutinins and lysins. Coca, likewise, failed to produce specific antihuman heterohemagglutinins and attributes these results to the presence of an antigenic complex common to all groups. Hooker and Anderson,⁵ however, have recently described the production of specific group hemagglutinins by some rabbits injected intravenously with small amounts of human corpuscles. These were demonstrated by absorption tests. No reasons were given for individual variation among rabbits in their response to injections with human cells. These investigators found that normal rabbit sera possess weak agglutinins for the four groups of human corpuscles and particularly for Group II and IV cells. Human sera likewise agglutinate rabbit corpuscles, but without group specificity. Group specific hemolysins for human corpuscles were found.

The Influence of Temperature Upon Hemagglutination.—Agglutination of corpuscles is usually a rapid phenomenon. With microscopic tests conducted at ordinary room temperatures agglutination is well marked within fifteen minutes and usually within a few minutes when large amounts of agglutinins are present. Macroscopic tests are usually conducted by incubation for an hour at 37° C., but agglutination occurs at lower temperatures equally well and very rapidly. Jervell⁶ has recently shown that the most pronounced agglutination occurs in the refrigerator and that absorption of agglutinin occurs more quickly and completely at low than at high temperature.

¹ Jour. Immunology, 1919, 4, 275.

² Centralbl. f. Bakteriol., 1899, 25, 546.

³ Münch. med. Wchn., 1899, 405.

⁴ Jour. Immunology, 1920, 5, 89.

⁵ Jour. Immunology, 1921, 6, 419.

⁶ Jour. Immunology, 1921, 6, 445.

MEDICOLEGAL APPLICATION OF HUMAN BLOOD GROUPING

In 1908 Epstein and Ottenberg¹ noticed that the groupings of human erythrocytes were hereditary and followed Mendel's law. In 1910 Von Dungern and Hirschfeld² examined 348 individuals belonging to 72 different families and proved the correctness of Ottenberg's observations. Ottenberg³ has recently reviewed the subject very carefully and concludes that blood grouping may prove of aid in determining parenthood in medico-legal cases involving the charge of illegitimacy.

Von Dungern and Hirschfeld have shown that the two agglutinogens A and B found in human corpuscles never occur in a child if not present in one of the parents; that when one of these is present in both parents it occurs in most of the children; when one (A or B) is present in only one parent, the child may inherit it; when neither parent has one of these substances (A or B) the child never shows it. Medicolegally, if A or B is present in a child's blood one of the alleged parents must possess it.

In determining the legitimacy of a child its blood and that of the mother and father are tested and grouped according to the Jansky classification. In cases of illegitimacy a fourth person is to be included, the alleged father or mother, as the case may be. The commonest instance, of course, is that of disputed paternity. According to Ottenberg's analysis of the work of Von Dungern and Hirschfeld, the results may be interpreted as follows:

1. If the woman and the man belong to Group I the child must be Group I to be theirs; if the child belongs to II, III, or IV it is not the child of the supposed father or mother, as the case may be.

2. If the woman is I and the man II or the woman is II and the man I the child must be I or II; if the child is III or IV it is not the child of the supposed father or mother, as the case may be.

3. If the woman is I and the man is III, or the woman is III and the man is I, the child must be I or III; if the child is II or IV it is not the child of the supposed father or mother, as the case may be.

4. If the woman is II and the man is II the child may be I or II; if the child is III or IV it is not the child of the supposed father or mother, as the case may be.

5. If the woman is III and the man is III the child may be I or III; if the child is II or IV it is not the child of the supposed father or mother, as the case may be.

The above unions comprise over 80 per cent. of all unions and are those on which the kind of possible offspring is definitely limited; they are the instances which may be of medicolegal value. The test, therefore, may not furnish evidence under the following two conditions:

6. All unions containing a member of Group IV and unions of II and III, may produce offspring of any of the four groups.

7. A child of Group I may result from any combination of parents.

If the child's blood is the correct group for the alleged parents, then the child *could* be their offspring, but not that it must be necessarily so. But, on the other hand, if the child's group is wrong for the alleged parents, then according to Ottenberg's analysis, one can say that the child must have a parent other than one of those asserted.

In infants and very young children the test can only be relied upon if it shows definite group characteristics. As stated above, the corpuscles of children acquire susceptibility to agglutination at about six months of

¹ Trans. New York Pathol. Soc., 1908, 8, 117.

² Ztschr. f. Immunitätsf., 1910, 6, 284.

³ Jour. Immunology, 1921, 6, 363; Jour. Amer. Med. Assoc., 1922, 78, 873.

age, which is considerably earlier than the appearance of agglutinins in their serum. Therefore this medicolegal test may not prove of value unless the child is at least six months of age; the test is apt to be most accurate when the child is two or more years of age.

Buchanan¹ has questioned this medicolegal application of blood grouping and states that on the basis of Mendelian laws, the only instances in which it appears that the blood group could be held as direct evidence would be in a family of four or more children of whom one was of a different group than the evident group represented in both parents, and all four grandparents. Buchanan states that a grandparent might be a heterozygote, in virtue of which she might transmit a character to a son or daughter, who in turn might be a heterozygote, and finally, in a family at issue the long-concealed character or group might appear. Gichner,² Learmouth,³ Keynes,⁴ Weszcesky,⁵ Tebbutt, and McConnel,⁶ however, have confirmed the hereditary nature of the blood group, and that when the group of the child and one parent is known, one may sometimes state to what group the other parent must belong. It is never possible, however, to say from the children alone to what group or groups the parents must belong.

For the Medicolegal Identification of Blood-stains.—There are no available means for differentiating human blood-stains, although by means of precipitin and complement-fixation tests a stain of human blood may be identified and differentiated from the bloods of the lower animals. However, if *fresh blood* is obtained *before drying* agglutination tests are of aid to this extent: if a blood-stain is from *one of two persons*, and it is found that these two individuals belong to *different* groups, typing the corpuscles of the blood-stain would indicate from which of the two persons the blood was derived; if the corpuscles are agglutinated by the serum of the accused individual, the blood could not be his own except in rare instances of auto-hemagglutination previously discussed. Dried blood-stains cannot be used because of hemolysis of the corpuscles.

TECHNIC OF TESTS FOR ISOHEMAGGLUTININS AND ISOHEMOLYSINS

As discussed in the chapter on Blood Transfusion the bloods employed should be compatible, that is, the blood of the donor should not agglutinate or hemolyze the corpuscles of the patient, and vice versa. It is generally agreed that tests for agglutinins alone are sufficient preliminary to blood transfusion on the basis of the assumption that when agglutinins are absent hemolysins are likewise.

However, in my experiments, I have found that hemolysins may be present in an occasional serum when the agglutinins are either entirely absent or present in such small amounts as to escape detection. For this reason I believe that tests for both agglutinins and hemolysins should be conducted, but of the two the former is much more important and the latter may be omitted. In addition to these tests the serum of the donor should be submitted to the Wassermann test if time and opportunity permit. The relation of syphilis to transfusion is discussed in the aforementioned chapter.

Macroscopic Test for Agglutinins and Hemolysins.—1. About 1 c.c. of blood is obtained from each donor from a prick of the finger in a small

¹ Jour. Amer. Med. Assoc., 1922, 78, 89; *ibid.*, 1922, 79, 180.

² Jour. Amer. Med. Assoc., 1922, 79, 2143.

³ Jour. Genetics, 1920, 10, 141.

⁴ Blood Transfusion, Oxford Med. Publications, 1921, 90.

⁵ Biochem. Ztschr., 1920, 107, 159.

⁶ Med. Jour. Australia, 1922, 1, 201.

tube containing 2 c.c. of a 1 per cent. sodium citrate in normal salt solution. An equal amount is collected in a small, dry test-tube; when coagulation has occurred the serum is separated, or secured by breaking up the clot and centrifuging.

2. From the recipient 1 c.c. of blood is placed in 2 c.c. of sodium citrate solution, and an equal amount is collected in a dry tube, allowed to coagulate, and the serum collected.

3. The sodium citrate tubes are centrifuged; the supernatant fluid is pipetted off, and the cells are washed again with normal salt solution. After the final washing enough normal salt solution is added to the sediment of cells to bring the total volume up to 1 c.c.

4. The serum tubes are also centrifuged, so that clear serums are obtained. These should preferably be free from hemoglobin stain.

5. *The following mixtures should be set up within twenty-four hours of the time of collecting blood in order that native complements may not have undergone deterioration.* Measurement may be made according to a drop from an ordinary 1 c.c. graduated pipet held vertically. Small sterile test-tubes (8 by 1 cm.) are to be used.

Tube 1: 4 drops of donor's serum + 1 drop of recipient's red-cell suspension.

Tube 2: 4 drops of recipient's serum + 1 drop of donor's red-cell suspension.

Tube 3: Control: 4 drops of donor's serum + 1 drop of donor's red-cell suspension. Should show no agglutination and no hemolysis.

Tube 4: Control: 4 drops of recipient's serum + 1 drop of recipient's red-cell suspension. Should show no agglutination or hemolysis.

Tube 5: Control: 1 drop of donor's red-cell suspension + 4 drops of normal salt solution. This serves as a control on the fragility of the corpuscles and isotonicity of the salt solution.

Tube 6: Control: 1 drop of recipient's red-cell suspension + 4 drops of saline solution.

One c.c. of salt solution is added to each tube and the tubes are gently shaken and placed in the incubator for two hours. They are to be inspected every half-hour. Agglutination is recognized macroscopically by the clumping of the red blood-cells into small masses which *cannot be easily broken up by agitation* and that later sink to the bottom of the tube as a small clot (Fig. 105). Hemolysis is likewise easily detected, as corpuscles tend to become hemolyzed within two hours. If doubt exists, the finer grades of hemolysis may be detected after the tubes have been allowed to stand overnight in an ice-chest, or at once by thorough centrifugalization. This method requires considerable serum and *weak agglutination may be overlooked.*

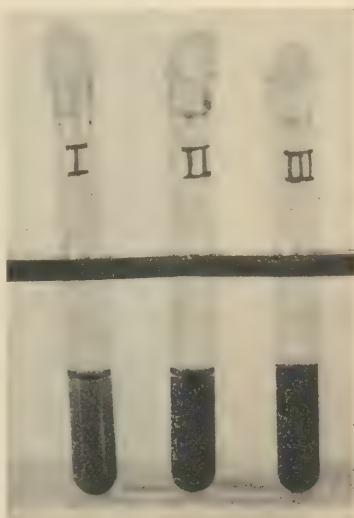


FIG. 105. —MACROSCOPIC HEMAGGLUTINATION.

The tube on the left shows agglutinated masses of corpuscles; the middle tube shows a negative reaction, and the tube on the right is a corpuscle control.

Microscopic Test for Hemagglutinins; Direct Method.—In this test no attempt is made to group the bloods; it simply consists of a direct test of the serum of the patient (recipient) for agglutinins for the corpuscles of each donor and of the serum of each donor for agglutinins for the corpuscles of the patient. Therefore, it does not require the preservation of group sera, is simple and efficient. Unger¹ has recently stated that even though the bloods of both recipient and donor are grouped, this direct test should be made in addition. I conduct the test as follows:

1. About 5 to 10 drops of blood are secured from a finger of the patient in a small test-tube carrying about 1 c.c. of 2 per cent. sodium citrate in physiologic saline solution. At the same time about 0.5 to 1 c.c. of blood is secured in a small dry test-tube for serum.

2. Blood is secured from each donor in the same manner.

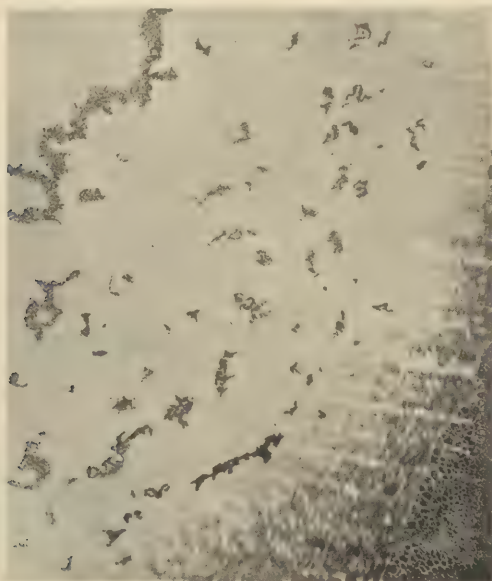


FIG. 106.—MICROSCOPIC HEMAGGLUTINATION.

Shows a positive agglutination of human corpuscles by human serum.

3. The suspensions of corpuscles are centrifuged and the supernatant fluids discarded; to the corpuscles in each tube is added about two volumes of saline solution and gently shaken. This step is not absolutely necessary as the suspension in citrate may be used.

4. The clots are gently broken up if necessary and centrifuged to secure a clear layer of serum in each tube.

5. The tests are now set up with cover-glasses and vaselined hanging drop slides as follows: (a) Two loopfuls (ordinary 4–6 mm. loop of platinum wire) of patient's serum is placed on a cover-glass; one loopful of donor's corpuscles are added, mixed, and the slide suspended. Similar slides are prepared with the corpuscles of each donor. (b) Two loopfuls of the donor's serum is placed on a cover-glass; one loopful of the patient's corpuscle suspension is added, mixed, and the slide suspended. Similar slides are prepared with the serum of each donor. (c) Two loopfuls of saline solution

¹ Jour. Amer. Med. Assoc., 1921, 76, 9.

are placed on a cover-glass; one loopful of patient's corpuscles are added, mixed, and the slide suspended. A similar preparation is made with the corpuscles of each donor. These are the corpuscle controls.

6. Each slide is examined microscopically (with $\frac{2}{3}$ objective) after standing fifteen minutes at room temperature. The controls are first inspected and should show no agglutination. As a general rule there is no difficulty in deciding upon the results; Fig. 106 shows a negative reaction and Fig. 107 a positive reaction. As shown in Fig. 108 the corpuscles at the margin of the slide may show a tendency to clumping or rouleaux formation from the effects of drying; these should not be mistaken for true agglutination.

Rous and Turner¹ have described a method similar to the above except that blood from patient and each donor is collected with a white corpuscle pipet and the tests set up in capillary tubes instead of test-tubes.

Microscopic Test for Hemagglutinins; Grouping of Blood.—For these tests it is necessary to have available known Group II and III sera; with these it is possible to determine to which of the four groups the blood of the



FIG. 107.—NEGATIVE HEMAGGLUTINATION REACTION.

patient belongs, and a corresponding or compatible donor belonging to the same group is selected for transfusion.

Preservation of the Agglutinating Sera.—A laboratory instituting these tests should secure small amounts of Group II and III sera from some other laboratory or from adults who have been reliably grouped.

These sera are best preserved in small ampules with 0.2 per cent. tricresol at a low temperature. Sanford² has described a method of preserving the sera by placing a loopful on each of a large number of cover-glasses, drying, wrapping in paper, and keeping in a refrigerator. Karsner and Koeckert,³ however, found that the agglutinins deteriorated during drying and that group specificity was lost after several weeks. Kolmer⁴ likewise found that with some sera drying on cover-glasses destroyed the agglutinins; for this reason only sera containing relatively large amounts of agglutinin should be used for Sanford's method, and it is well to test several glasses a few days after drying to make sure that agglutinins are present. With these precautions the method may be satisfactory; I have not clearly

¹ Jour. Amer. Med. Assoc., 1915, 64, 1980.

³ Jour. Amer. Med. Assoc., 1919, 74, 1207.

² Jour. Amer. Med. Assoc., 1918, 70, 122.

⁴ Jour. Amer. Med. Assoc., 1919, 73, 1459.

observed the loss of group specificity described by Karsner and Koeckert, although it may be that I did not keep my preparations sufficiently long under observation. Holt and Reynolds¹ have recently stated that the hemagglutinins are contained in the pseudoglobulin fraction of human serum, none being found in the euglobulin and albumin fractions. Desiccated pseudoglobulins were found to retain agglutinating properties for two and a half months. At any rate, I am quite sure that preservation of the sera in fluid form as described is just as simple and probably much better.

After one secures a little Group II and III sera for starting, an ample supply of each is easily provided for future use. It is my practice to type the bloods sent to the laboratory for the Wassermann test, and secure sera in this manner. After drawing off the sera from a number of specimens, corpuscles from each are easily secured, and the group to which each belongs determined by the method described below. For example, if the corpuscles

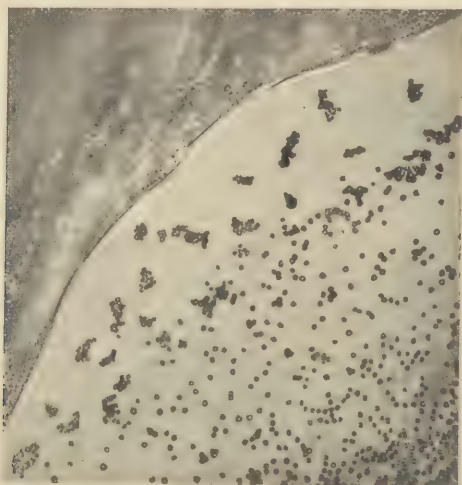


FIG. 108.—FALSE CLUMPING AND ROULEAUX FORMATION DUE TO DRYING.

of an individual belong to Group II the serum does likewise and is preserved with tricresol in a refrigerator. In this manner fresh Group II and III sera are obtained at least once a month; even Group I and IV sera may be secured and kept in the same manner for controls, although these are not absolutely necessary.

It is necessary to depend upon human sera; immune sera cannot be prepared. Kolmer and Trist² have immunized rabbits with corpuscles belonging to the four groups; the immune sera agglutinated the corpuscles of the corresponding group slightly better than the corpuscles of other groups, but not sufficiently well to permit the use of such sera for grouping. In other words, rabbits injected with Group II corpuscles produced agglutinins for the corpuscles of all four groups, but slightly better for Group II; absorption of such sera with Group I, III, and IV corpuscles removed all agglutinin so that such sera could not be purified.

Technic of Tests.—The following method is essentially similar to that

¹ Jour. Amer. Med. Assoc., 1922, 79, 1684.

² Jour. Immunology, 1920, 5, 89.

described by Brem¹ and Lee² and found very useful during the war, especially by Karsner.³

1. From 0.5 to 1 c.c. of 1 per cent. sodium citrate in physiologic saline solution is placed in a small test-tube; 2 or 3 drops of the blood to be tested are dropped in, secured by pricking the finger.

2. Three cover-glasses are cleaned; upon No. 1 are placed 2 loopfuls of saline solution; upon No. 2, 2 loopfuls of Group II serum, and upon No. 3, 2 loopfuls of Group III serum. One loopful of corpuscle suspension is added and mixed with the fluid on each slide. No. 1 is the corpuscle control.

3. Each slide is inverted in position on a vaselined Widal hanging drop slide, and examined microscopically with a $\frac{2}{3}$ objective after standing fifteen minutes. The control (No. 1) should show no agglutination; a negative reaction is shown in Fig. 107, and due care must be exercised against regarding rouleaux formation as agglutination. A positive reaction is shown in Fig. 106, and is usually easily read.

4. The grouping is made with slides No. 2 and 3 as follows:

(a) No agglutination in either slide: The blood belongs to Group I of the Jansky classification or Group IV of the Moss classification.

(b) Agglutination in both slides: The blood belongs to Group IV of the Jansky classification, or Group I of the Moss classification.

(c) Agglutination with Group II serum; no agglutination with Group III serum: the blood belongs to Group III in both the Jansky and Moss classifications.

(d) Agglutination with Group III serum; no agglutination with Group II serum: The blood belongs to Group II in both the Jansky and Moss classifications.

Since both the Jansky and Moss classifications are in use, the worker must state in his report the classification employed; otherwise there is danger, if a donor has been grouped according to one method and the patient by the second, of confusing Group I and Group IV bloods. As previously stated, the Jansky classification has been recommended (purely on the basis of priority) for general adoption in order to avoid this confusion.

Vincent's Open Slide Method.—Vincent⁴ has described a simple slide method which Ottenberg⁵ has found very satisfactory:

1. One drop of Group II serum is placed on the left-hand side of a slide and 1 drop of Group III serum on the right-hand side.

2. The finger or ear of the patient is pricked and a small drop of blood transferred by the knife or lancet to the Group II serum and mixed; the instrument is now cleansed and blood transferred to the Group III serum; or 2 to 3 drops of blood may be collected in 1 c.c. of 2 per cent. sodium citrate solution as described above, and a drop added to each of the two sera.

3. The slide is tilted and rotated gently, so that the cells are uniformly distributed; this is repeated every few minutes. Agglutination is easily seen with the naked eye in from one to ten minutes at room temperature. Rouleaux formation usually appears more slowly than agglutination, and the clumps are broken up by rocking the slide, which increases agglutination. In doubtful cases the reading should be confirmed by microscopic examination.

As suggested by Culpepper and Ableson,⁶ the dried tests may be kept as permanent records. According to Ottenberg, false agglutination due to drying does not occur if the slides are not moved after the first ten or fifteen minutes, and chipping of the dried specimens may be prevented by painting them with a layer of collodion.

¹ Jour. Amer. Med. Assoc., 1916, 67, 191.

² Brit. Med. Jour., 1917, 2, 684.

³ Jour. Amer. Med. Assoc., 1918, 70, 769.

⁴ Jour. Amer. Med. Assoc., 1918, 70, 1219.

⁵ Jour. Amer. Med. Assoc., 1922, 79, 2137.

⁶ Jour. Lab. and Clin. Med., 1921, 6, 276.

CHAPTER XVII

PRECIPITINS

CLOSELY allied to the agglutinins are antibodies known as *precipitins*. They act on dissolved albuminous bodies in a manner quite similar to the action of agglutinins upon formed cellular elements. For example: (1) If typhoid immune serum is added to a bouillon culture of typhoid bacilli agglutination occurs; (2) if the culture is filtered and the immune serum is added to the clear sterile filtrate cloudiness appears and finally a precipitate forms. The first is an example of the action of agglutinins upon the formed bacilli, and the second illustrates the action of precipitins upon the albumins of dead and dissolved bacilli.

Precipitins are formed not only for bacterial albumins, but for most any soluble animal (*zoöprecipitin*) and vegetable protein (*phytoprecipitin*) as well. If the serum of a rabbit immunized with horse-serum is added to horse-serum a precipitate forms owing to the presence of a specific precipitin in the immune serum. Normal rabbit-serum does not possess this power.

Definition.—*The precipitins are specific antibodies that develop in the serum of animals inoculated with bacteria or with solutions of animal or vegetable albumins which possess the power of producing a precipitate in a clear solution of the particular albumin or culture filtrate against which the animal has been immunized.*

Historic.—Kraus¹ was the first to study and describe the bacterial precipitins. He observed that when the serums of animals that have been immunized against cholera, typhoid, or plague are added to a clear filtrate of the respective bouillon cultures of their bacteria, instead of to the bacteria themselves, the clear solution becomes turbid and a precipitate forms.

This reaction was found to be quite specific, *i. e.*, it occurs best with the filtrate of the homologous bacteria, and to a much less extent with closely allied species. For example, the typhoid immune serum does not produce a precipitate with a filtrate of *Spirillum cholerae*, and similarly a cholera immune serum does not produce a precipitate with the filtrate of *Bacillus typhosus*. Kraus advocated the precipitin reaction as a means of identifying and differentiating certain species of bacteria, but the test possesses no advantage for these purposes over agglutination reactions and is not generally employed.

Tchistovitch² was the first to call attention to the non-bacterial precipitins. This observer found that the serum of rabbits inoculated with eel-serum, when mixed with a small quantity of the eel-serum, caused a precipitate to form.

About the same time (1899) Bordet found that the serum of rabbits inoculated with the serum of chickens, when mixed with the chicken-serum, gave a specific precipitate. A little later Bordet³ produced an antimilk immune serum (*lactoserum*) by inoculating rabbits intraperitoneally with milk partially sterilized by heating to 65° C. When this immune serum was mixed with the homologous milk, small particles appeared, which gradually formed larger flakes and sank to the bottom of the fluid. It was

¹ Wien. klin. Wchn., 1897, 10, 736.

² Ann. d. l'Inst. Pasteur, 1899, 13, 413.

³ Ann. d. l'Inst. Pasteur, 1899, 13, 240.

found that the lactoserums were specific, *i. e.*, cow lactosera would precipitate only cow casein, human sera only human casein, etc.

Wladimiroff was the first to use the bacterial precipitin reaction as a practical diagnostic test. He showed that the serum of a horse suffering from glanders would, when added to a clear filtrate of a culture of *Bacillus mallei*, produce a precipitate. The technic of these reactions is, however, more difficult than with the agglutination tests, and as the reactions are usually not more delicate or more advantageous than the latter, they are seldom employed.

Wassermann¹ and Schütze² made a very important practical demonstration of the value of serum precipitins in differentiating the blood and secretions of man and animals. For example, the serum of rabbits immunized with various bloods would react with solutions of old and dried specimens of their respective bloods, and although "group" precipitins were found present in the tests with the blood of closely allied species, yet the value of the reaction was not impaired to any extent when a proper technic, with correct dilutions, was employed. These discoveries were found to possess considerable value in forensic medicine, particularly in the recognition of the source of blood-stains.

Nuttall,³ in a thorough and painstaking research with the blood from 500 animals, was able to study the "blood relationship" of various animals as based upon group precipitins. For example, the serum of a rabbit immunized with human blood will react best with human serum, then with the sera of the higher apes, and finally with the lower orders of monkeys. Similar reactions were found to occur among the lower animals.

Nomenclature.—The antibody in an immune serum responsible for the phenomenon of precipitation is called *precipitin*; the substance or antigen responsible for the production of this antibody is known as the *precipitinogen*; the *precipitate* is the end-product of the reaction between precipitinogen and precipitin. Just as toxoids and agglutinoids may be formed, so precipitin may be modified to *precipitoid*.

Kinds of Precipitins.—Precipitins may be classed into two broad groups, namely, *phytoprecipitins*, for the albumins of plants, and *zoöprecipitins*, for the albumins of animals.

In the group of plant precipitins are included those for bacteria, yeasts, fungi, and higher plants:

Bacterioprecipitins include those found in the sera of animals normally or after immunization with bacteria, which act upon culture filtrates of the corresponding bacteria or upon a solution of the substance of these microorganisms. They will be discussed in more detail later in this chapter.

Mycoprecipitins include those for the albumins of yeasts and fungi. Schütze⁴ claims to have produced them experimentally by injecting rabbits with the dissolved albumins of top and bottom beer yeasts, grain, and potato yeasts, prepared by rubbing up cultures in a mortar in sterile 25 per cent. soda solution with the addition of powdered glass and sand. A clear fluid was obtained by centrifuging, which was used for immunization and for the conduct of the tests; all of these yeasts reacted to the various antisera.

Precipitins for the albumins of higher plants have also been produced experimentally probably first by Kowarski,⁵ who immunized rabbits with

¹ See article on Glanders in Kolle and Wassermann's *Handbuch*, vol. 5, 2d ed.

² *Ztschr. f. Hyg.*, 1901, 36, 5; *Berl. klin. Wchn.*, 1901, 38, 187.

³ *Blood Immunity and Blood Relationship*, Cambridge University Press, 1904.

⁴ *Ztschr. f. Hyg.*, 1901, 38, 493.

⁵ *Deutsch. med. Wchn.*, 1901, 27, 442.

solutions of non-coagulable proteins from wheat flour and obtained a precipitin for a saline extract of wheat flour. Weaker reactions were observed with extracts of the seeds of peas, rye, and barley, but not with oats.¹

In the group of zoöprecipitins are included those for milk, sera, bloods, inflammatory exudates, and transudates and meats.

Lactoprecipitins or *lactosera*, first discovered by Bordet,² occur in immune sera and precipitate milk casein. They will be discussed later.

Hematoprecipitins include those for plasma, sera, and extracts of dried blood. Most investigations have been conducted with this large group and they have been found of great practical value and interest.

In addition to these, precipitins have been produced for the albumins of meats, bones, egg-white, honey, cerebrospinal fluid, pleural exudates, urine, and other substances to which reference will be made later.

The precipitins derive their names from their precipitinogens, as, for example, a precipitin produced by injecting rabbits with ox-serum is designated anti-ox precipitin.

Normal (Natural) Precipitins.—Although agglutinins may be found in normal serum, it is decidedly uncommon to find *normal precipitins*. By using low dilutions of normal sera Noguchi³ has demonstrated normal precipitins in the blood of some of the cold-blooded animals. Ascoli⁴ has found ox-serum to contain precipitins for the sera of man, dog, pig, goat, rabbit, guinea-pig, and fowl; dog-serum to contain precipitins for the serum of the fowl and for egg-white; goat-serum for the sera of fowl and guinea-pig. These normal precipitins are very important when attempting to identify a blood, and may lead to error unless the specific precipitating antiserum is sufficiently powerful to work with the highest possible dilutions of the blood extract.

Isoprecipitins have also been found by Ascoli, but are very rare; these are precipitins in the serum of an animal for the serum of another animal of the same species. Schütze⁵ claims to have produced these precipitins in 2 rabbits by injecting rabbit-serum into 32 rabbits; he states that he found it more convenient to obtain isoprecipitins from goats treated with injections of goat milk over a period of a month or longer.

Immune Precipitins.—These are ordinarily produced by immunization of animals with a foreign albumin; in man they may occur during the course of a bacterial infection or as the result of injections of bacterial vaccines, or the serum of the horse or other animal.

Nature and Properties of Precipitins.—According to the side-chain theory, precipitins are antibodies or *receptors* of the *second order*, composed of a combining arm or haptophore group for the precipitinogen, and a zymophore or precipitinophore group that precipitates the antigen. Their structure is, therefore, seen to be quite similar to that of agglutinin, the difference being largely due to the different functions of the zymophore group.

The properties of precipitins are quite similar to those of agglutinins. They are fairly resistant bodies, resist the effect of drying for prolonged periods, but are gradually destroyed by heating to 60° to 70° C. When inactivated by exposure or heat, they cannot be reactivated by the addition of fresh normal serum, and, therefore, they bear no relation to the complements.

¹ Literature reviewed by Wells and Osborne, Jour. Infect. Dis., 1911, 8, 66.

² Ann. d. l'Inst. Pasteur, 1899, 13, 225.

³ Univ. of Pennsylvania Med. Bull., 1902.

⁴ Münch. med. Wchn., 1903, No. 5.

⁵ Deutsch. med. Wchn., 1901, 28, 4; *ibid.*, 804.

In their *chemical nature* precipitins are thrown down in the globulin fractions of the serum, although there is not a uniformity of opinion on the particular fraction carrying these antibodies. For example, Leblanc¹ claims to have found them in the pseudoglobulins, while Eisenberg, Obermayer, and Pick² identified them with the euglobulin fraction of immune serum, and this is the view most generally accepted. They are slowly destroyed by tryptic digestion, more slowly by pepsin.

Precipitoids.—The haptophore or combining arm of precipitin is quite stable; the precipitophore group is more labile, and is affected by heat, and when this less resistant arm is lost, the receptor is called a *precipitoid*. Like agglutinoids, the precipitoids are of practical interest from the fact that their haptophore arm will not only combine with precipitinogen, but displays a greater activity in this direction than the whole receptor or precipitin itself, and when union between precipitinogen and precipitoid has occurred, precipitation does not result. Hence in low dilutions of a precipitin serum the phenomenon of precipitation is slight or altogether absent, whereas in higher dilutions the reaction becomes evident.

The precipitoids were first described by Müller,³ who heated a lactoserum at 70° C. and found that it prevented precipitation when added to unheated and active lactoserum. They have been produced by Kraus and von Pirquet⁴ by heating typhoid and cholera bacterioprecipitins at 58° C.; these investigators also found them in old antisera. These sera cannot be activated by the addition of fresh serum, and the precipitoids are chiefly of interest in connection with the practical applications of the precipitin reaction, due care being necessary to avoid their neutralizing effect by using the precipitin sera in progressive dilutions. The mechanism of this phenomenon is very interesting and practically important. It finds a close analogy in the so-called protective colloids, the addition of precipitoid serum to a mixture of precipitin and antigen and the consequent prevention of precipitation being likened to the prevention of precipitation of mastic by normal serum by the addition of heated serum; however, this does not explain the specificity of the process. The prevention of precipitation of colloids by heated serum is non-specific but precipitoids are highly specific, that is, will only prevent the action of the corresponding precipitins. As shown by Welsh and Chapman,⁵ heating an immune serum from which the precipitin has been removed does not result in the formation of precipitoids. According to these observers heated immune serum (precipitoid) prevents precipitation by active precipitin, and antigen by specifically dissolving the precipitate which would otherwise make its appearance.

Antiprecipitins.—These possess the power of neutralizing the precipitins, but occur in fresh unheated sera and should not be confused with the precipitoids. Nuttall⁶ believes that they may occur in normal sera (natural antiprecipitins), although it is possible that the neutralizing effects of normal serum upon precipitation may be due to the solution of the precipitin in its homologous serum.

Immune antiprecipitins have been produced by Kraus and Eisenberg⁷ by treating goats with goat lactoserum obtained by injecting rabbits with goat's milk; antilactoserum has also been prepared by Schütze.⁸

¹ La cellule, 1901, 18, 337.

² Blood Immunity and Relationship, 96, 97.

³ Münch. med. Wchn., 1902, xlv, 1330.

⁴ Centralbl. f. Bakteriöl., 1902, 27, 60.

⁵ Jour. Path. and Bacteriol., 1909, 13, 206.

⁶ Blood Immunity and Relationship, p. 149.

⁷ Wien. klin. Wchn., 1902, 27, 212.

⁸ Deutsch. med. Wchn., 1901, 28, 4.

Group Precipitins.—These are not as prominent as group agglutinins, yet they are formed to a certain degree and are of much practical importance in attempting to differentiate bacteria and serums by the precipitation method. Although precipitins are highly specific, the principle of serum dilution, as emphasized under Agglutination, must be closely observed in order to dilute the group precipitins to such small amounts as to prevent them from interfering with the chief precipitin. This principle is of particular importance in differentiating the bloods of various animals, and especially in medicolegal cases, where the precipitin reactions are employed for the diagnosis of blood-stains. From both a theoretic and practical standpoint *these group precipitins are of considerable importance*, and I shall discuss them more fully later in a consideration of the specificity of precipitins.

Origin of Precipitins.—Kraus and Levaditi¹ have assigned the leukocytes as the chief cells concerned in the production of precipitins; Kraus and Scheffmann² have expressed a similar claim for the vascular endothelium. Cantaguzene³ believes that these antibodies are formed chiefly in the lymphoid tissues and bone-marrow, and particularly by the mononuclear macrophages. The results of studies on the influence of x-rays upon precipitin production by Benjamin and Sluka,⁴ and upon antibody production in general by Hektoen,⁵ have shown that any agent that injures bone-marrow and lymphoid tissues reduces antibody production, indicating the active participation of these tissues in antibody production.

Production of Precipitins.—Immune serums for diagnostic purposes are produced by injecting the precipitinogenous fluid into the veins, peritoneal cavity, or subcutaneous tissues of animals, usually rabbits.

As in the case of agglutinin formation, not all animals possess equally the power of forming a precipitin for a given albumin. Rabbits are generally employed; guinea-pigs produce unsatisfactory sera. While this point is of general interest with the bacterioprecipitins, it becomes of particular importance in relation to serum precipitins. For example, an animal will not form a precipitin active against its own serum. If formed, it would be an aut precipitin, or *isoprecipitin*, and, as a rule, animals do not form antibodies for their own tissue constituents. Furthermore, animals are unlikely to form precipitins for the proteins of other members of the same species, or if precipitins are produced, they are usually the result of prolonged immunization of a number of animals. Precipitin formation is also slight for the proteins of other animals that are closely related either zoologically or biologically. For example, attempts at immunization of a guinea-pig with the serum of a rabbit, a pigeon with that of a hen, or a monkey with human serum, are procedures that do not usually yield good precipitating serums. The technic of preparing various precipitins is described later in this chapter under Practical Applications.

Chemistry of Precipitinogens.—Only proteins are known to possess the property of inducing the formation of precipitins. Furthermore, they must be *foreign proteins* as previously stated. Almost any vegetable or animal protein may act as a precipitinogen under these conditions; of the serum proteins the globulins are apparently more active than the albumins.

Obermayer and Pick⁶ and Jacoby⁷ claim to have produced non-protein

¹ G. r. Acad. d. Sci., 1904, 4, 5.

² Ann. d. l'Inst. Pasteur, 1906, 20, 225.

³ Ann. d. l'Inst. Pasteur, 1908, 22, 54.

⁴ Wien. klin. Wchn., 1908, 21, 311.

⁵ Jour. Infect. Dis., 1915, 17, 415; *ibid.*, 1918, 22, 561.

⁶ Wien. klin. Wchn., 1904, 265.

⁷ Hofmeister's Beiträge, 1901, vol. i.

antigens by digesting egg-white and ricin respectively, to the stage when they no longer gave the usual protein reactions; Myers¹ claims to have produced specific precipitins with Witte's peptone, but Fink² and others have failed to produce precipitins with these proteoses. Kurt Meyer³ claims to have produced a precipitin for lecithin (lipoid precipitin), but these findings have not been confirmed. It is now the consensus of opinion that only the protein molecule can induce the production of precipitins, and that protein-free lipoids and proteins split to the peptones or further are not antigenic.

Purified proteins as caseinogen may act as precipitinogens as well as mixtures of proteins. Some of the fractions of protein cleavage are likewise antigenic, and Corin⁴ considers the paraglobulins of serum to be the active seroprecipitinogens.

As previously stated, precipitins are rarely produced by injecting an animal with its own serum or with the serum of another animal of the same species. As shown by Obermayer and Pick,⁵ however, rabbit serum treated with iodine or nitric acid are so altered that when these are injected into rabbits precipitins are produced; these precipitins reacted only with iodized protein and nitroprotein respectively, but not only with iodized and nitroproteins of rabbit serum, but of beef serum as well. In other words, the precipitins were no longer specific for the species.

Coctoprecipitins and Thermoprecipitinogens.—The precipitin produced by injecting an animal with a heated precipitinogen as heated or boiled serum, is called a coctoprecipitin. Precipitinogens are resistant to moderate heating and heated extracts of bacteria are commonly used for precipitin tests under the term *thermoprecipitins*; this, however, is a misnomer and such heated extracts should be designated as *thermoprecipitinogens*. In other words, thermoprecipitinogens produce coctoprecipitins, the former being the special name applied to the antigen and the latter to the antibody.

This question of heated antigen possesses a great deal of academic interest as well as practical importance, the latter in reference to the detection of adulteration of sausages with heated dog and cat flesh.

Heating to coagulation does not destroy the precipitinogenic properties of a protein-like serum. Obermayer and Pick⁶ found that beef-serum boiled for a short time served to induce the production of a precipitin (coctoprecipitin) which reacted with both unheated and boiled antigens; on the other hand, they found that precipitin induced by injecting rabbits with unheated serum reacted only with unheated serum and not with heated serum.

Schmidt,⁷ who has studied this subject with particular care, found that the coctoprecipitin induced by injecting rabbits with serum heated at 70° C. for thirty minutes reacted well with a boiled antigen, and in practical tests for the detection of boiled meats advises this method for preparing the immune serum. He also found that antigens heated at 70° C. for thirty to sixty minutes are precipitable by precipitins, but to a lesser extent than unheated antigen. Fornet and Müller⁸ have found the coctoprecipitins non-specific, that is, the precipitins induced by injecting rabbits with heated

¹ Centralbl. f. Bakteriöl., Abt., 1900, 28, 237, 244.

² Jour. Infect. Dis., 1919, 25, 97.

³ Ztschr. f. Immunitätsf., orig., 1913, 19, 313.

⁴ Deutsch. med. Wchn., 1902, 136.

⁵ Wien. klin. Wchn., 1906, 12.

⁶ Wien. klin. Wchn., 1906, 12.

⁷ Ztschr. f. Immunitätsf., orig., 1912, 13, 166.

⁸ Ztschr. f. Hygiene, 1910, 66.

muscle extracts reacted not only with the homologous antigen, but with other foreign proteins as well; similar results have been reported by Zinsser and Ottenberg¹ who injected rabbits with sera boiled for three to five minutes, and found that the precipitins acted upon boiled antigens, but were not specific.

Since the precipitins (coctoprecipitins) induced by injecting animals with boiled antigens (thermoprecipitinogens) are non-specific it is necessary to prepare immune sera for the detection of boiled meats by injecting sera or meat extracts (preferably the former) heated at 70° C. for thirty minutes (Schmidt's "70° C. precipitins"), or to rely upon the use of extra powerful precipitins produced by injecting unheated serum (Fornet and Müller).

Mechanism of Precipitation.—Of the various theories advanced to explain the phenomenon of precipitation, none has received so much support experimentally as that regarding the reaction a colloidal phenomenon, first advanced by Landsteiner,² and advocated by Bordet, Porges, Neisser, and Friedemann, Gengou, and others in explanation of agglutination.

Both the precipitinogen and precipitin are colloids and closely follow the laws governing colloidal reactions. Electrolytes must be present in the form of salts and so alter the electric state of colloidal particles that their surface tension is decreased, and as a result of this change neighboring particles coalesce in such quantities as to produce a visible precipitate. Salts are likewise necessary for serum precipitation, and there is a close analogy between serum and colloidal precipitation.

As stated by Krogh,³ however, we are not justified in assuming that specific serum precipitation is one solely of colloidal chemistry, but rather that colloidal phenomena are to be regarded as preliminary to a real chemical process that completes the reaction and gives it specific characters.

The apparent *coexistence of both antigen and antibody* in antiserum is sometimes encountered, and especially when rabbits are rapidly immunized with large doses of serum and bled within a week or ten days after the last injection. This phenomenon is shown by the fact that the immune serum may be clear but show precipitation when mixed with dilutions of antigen (indicating the presence of precipitin) or when added to homologous precipitating sera (indicating the presence of antigen). This paradoxical phenomenon of the presence side by side in the same serum of precipitinogen and precipitin, but incapable of reacting with each other, has been recorded by Linossier and Lemoine,⁴ Eisenberg,⁵ Ascoli,⁶ von Dungern,⁷ Gay and Rusk,⁸ Zinsser and Young,⁹ Weil,¹⁰ and others.

Several explanations have been offered. In the first place von Dungern has doubted the actual coexistence of antigen and antibody in the same serum and believes that the apparent presence of the two side by side is due to the fact that complex antigens, as horse-serum, which have been used for immunizing rabbits in most experiments, contain several antigenic substances stimulating the production of several partial precipitins. In sera in which antigen and precipitin are found apparently side by side and

¹ Proc. New York Path. Soc., 1914.

² Centralbl. f. Bakteriöl., orig., 1906, 41, 108; *ibid.*, 1906, 42, 353.

³ Jour. Infect. Dis., 1916, 19, 452.

⁴ C. R. de la Soc. de Biol., 1902, 54, 85.

⁵ Centralbl. f. Bakteriöl., 1902, 31, 773.

⁶ Münch. med. Wchn., 1902, 39, 1409.

⁷ Centralbl. f. Bakteriöl., 1903, 34, 355.

⁸ Univ. of Calif. Publ. Path., 1912, vol. ii.

⁹ Jour. Exper. Med., 1913, 17, 396.

¹⁰ Jour. Immunology, 1916, 1, 19, 35, 47.

free, he believes that the antigen is of a nature that has no affinity for the particular partial precipitin present with it. Zinsser and Young have not accepted this view and believe that antigen and specific antibody may co-exist in the same serum, precipitation being prevented by protective colloids. Weil, however, working with horse-serum as a complex antigen and crystalline egg-albumen as a purified antigen, has adopted the views of von Dungern, having found that with the latter antigen partial precipitins are not produced and the antisera do not present the paradoxical phenomenon of free precipitinogen and precipitin existing side by side. The question, however, is still an open one requiring further investigation. In the living animal antigen and antibody may be present in the serum, probably in loose combination and easily dissociated in the test-tube by the addition of fresh antigen whereby precipitin becomes available and precipitation results.

The *origin of the precipitate* formed during the reaction is of interest. When a very potent immune serum is employed, the precipitinogen is so highly diluted that it no longer gives any of the chemical reactions for proteins, but when the precipitating serum is added it may yield, nevertheless, a heavy precipitate. The precipitate can, therefore, hardly be regarded as due to the slight trace of albumin in the precipitinogen, and, furthermore, if the precipitating serum is diluted, the precipitate becomes smaller and smaller, and if the dilution is increased, it finally disappears altogether. For this reason the precipitate is generally considered as originating mainly in the immune serum as indicated especially by the work of Welsh and Chapman,¹ being the insoluble modification of the previously soluble proteins of the precipitin.

However, when the reaction is maximum, a portion of the precipitate is apparently derived from the antigen. Wells² states that the precipitate may contain more nitrogen than corresponds to the entire euglobulin fraction of the immune serum which carries the precipitin; however, it is always less in amount than the total globulin of the immune serum. Furthermore, as shown by Weil,³ the precipitate is able to sensitize guinea-pigs anaphylactically in both an active and passive manner indicating the co-existence of both antigen and antibody. The serum precipitinogen has been recovered by Weil by treating the precipitate with salt solution and solutions of sodium carbonate; these procedures, however, did not dissociate precipitin. Extraction with trypsin and leukocytes yielded both antigen and antibody.

The precipitate is readily dissolved in weak acids and alkalis, has the power of binding complement as shown by Gay,⁴ and when digested or split, produces poisonous substances designated by Friedeberger as anaphylatoxins.

Specificity of Precipitins.—The high specificity of the precipitins is attested to by a very large number of investigations with different protein substances. The bacterioprecipitins may react with extracts of closely related bacterial species in exactly the same manner as the agglutinins, but in practical work the influence of these group precipitins may be avoided by using varying amounts of antiserum in a manner analogous to the agglutination reaction. Furthermore, the group precipitins are removable from immune serum by absorption methods as are the group agglutinins.

¹ Proc. Roy. Soc., 1908, 80, 161; Ztschr. f. Immunitätsf., 1911, 9, 517.

² Chemical Pathology, 4th ed., 186.

³ Jour. Immunology, 1916, 1, 35.

⁴ Univ. of Calif. Publ. Path., 1911, 2, 1.

An antiserum obtained by immunizing a rabbit with human serum may react not only with human serum, but likewise with the sera or blood extracts of the higher apes. This is shown by the extensive investigations of Nuttall¹ upon this subject, the reactions becoming weaker as the species examined is farther removed from man zoologically, when the sera were tested with five different antihuman sera:

34 Specimens of human blood (4 races) gave 100 per cent. precipitate.

8 Simudæ (3 species) gave 100 per cent. precipitate.

36 Cercopithecidæ (26 species) gave 92 per cent. precipitate.

13 Cebidæ (9 species) gave 73 per cent. precipitate.

4 Hapalidæ (3 species) gave 50 per cent. precipitate.

2 Lemuroidea (2 species) gave 0 precipitate.

These investigations by Nuttall, covering 16,000 tests, have proved very valuable for the study of blood relationships not only of the primates, but of relationships in the families of other carnivora, as the dog, cat, hyena, etc.; likewise among the insectivora, rodentia, ungulata, reptilia, aves, etc. Among the primates antihuman serum is most likely to react with the blood of the chimpanzee, the different members of the Simudæ reacting as follows when their sera were tested with antihuman serum:

Chimpanzee gave 100 per cent. precipitate.

Gorilla gave 64 per cent. precipitate.

Ourang-outang gave 42 per cent. precipitate.

Antihuman sera may also react very slightly with 2 to 24 per cent. of the sera of other carnivora, rodents, and the lower animals in general when the tests are made with low dilutions of antigen, due to the presence of natural precipitins or group precipitins; as shown by Uhlenhuth and Weidanz,² however, these non-specific reactions may be avoided by using higher dilutions of antigen, experience having shown that with 1 : 1000 dilutions of antigen the reactions are absolutely specific. This is well shown in the following table by Hektoen³:

SPECIFICITY OF PRECIPITIN IN SERUM OF RABBIT INJECTED WITH HUMAN BLOOD

Blood.	Highest dilution giving precipitate with antihuman serum after twenty minutes at room temperature.
Fish.....	1 : 10
Chicken.....	1 : 10
Rabbit.....	0
Guinea-pig.....	1 : 10
Rat.....	1 : 10
Cat.....	1 : 10
Dog.....	1 : 10
Swine.....	1 : 10
Sheep.....	1 : 10
Beef.....	1 : 10
Horse.....	1 : 10
Goat.....	1 : 10
Monkey (Macacus rhesus).....	1 : 100
Human.....	1 : 5000

Recently attention has been focused again on these cross reactions and particularly in connection with medicolegal work. Years ago Nuttall pointed out that precipitin reactions with the sera of animals which had

¹ Blood Immunity and Blood Relationship, Cambridge University Press, 1904, 165.

² Praktische Anleitung zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens, Fischer, Jena, 1909.

³ Jour. Amer. Med. Assoc., 1918, 70, 1275; Jour. Infect. Dis., 1922, 31, 72.

received many injections of a foreign protein were not strictly specific for that protein, as the antibodies produced reacted with the proteins of other animals of the same group or of the same class. Nuttall called this, for instance, the "mammalian reaction." In spite of this, however, the titration of the precipitin has shown sufficient quantitative differences to allow specific distinctions to appear. Lately, since Forssmann's discovery of heterophile antigens (discussed on page 159), more doubt has been thrown upon the validity of the precipitin reactions.

Thus Friedberger and Jarre¹ found that comparative tests with the sera of rabbits immunized to the blood proteins of various animals showed group precipitin reactions which cannot be explained by a biologic relationship of these animals. A rabbit immunized to horse-serum developed precipitins also for the serum proteins of ox, deer, goat, pig, sheep, and man. An antidog-serum from a rabbit precipitated horse and donkey proteins.

These antisera were next absorbed with the red corpuscles and other organ cells of guinea-pig and sheep. The sera, first inactivated, were mixed with a suspension of cells which had been heated to 100° C. After fifteen minutes of incubation of the serum and heated cells at 37° C., all the heterologous precipitins were absorbed, and were removed with the sediment of cells after centrifugalization. An antihorse-serum from a rabbit, which before treatment with sheep corpuscles precipitated ox-, sheep-, goat-, and pig-serum, after absorption with sheep cells contained only precipitin for horse-serum. Absorption with a heterologous antigen, therefore, rendered the serum strictly specific for its homologous antigen, as all the group antibodies were removed.

This work, however, has not been confirmed by Manteufel and Beger.² They found that 63 per cent. of all precipitating sera, active against human, beef, and other proteins, were absolutely specific, that 24 per cent. gave some precipitate with heterologous antigens diluted 1 : 100, and that 13 per cent. precipitated heterologous antigens diluted 1 : 1000. All these sera, however, were practically useful, as their titers for their homologous antigens were high, 1 : 10,000 or 1 : 20,000. The longer the course of immunization of an animal, the less specific the serum produced.

They found also that the formation of heterologous precipitin is not the same process as the formation of the heterogenetic hemolytic amboceptor which occurs when certain animals are injected with extracts from the organs of other animals as described by Forssmann.

Attempts were made to render sera strictly specific by absorbing from them the heterogenetic antibody with sheep corpuscles, as reported by Friedberger and his associates. These experiments, as well as other experiments on the absorption of precipitins with solutions of proteins, were unsuccessful.

These experiments emphasize, therefore, the great importance of group precipitins and particularly in medicolegal work; also the necessity for using highly potent and properly titrated sera and an extremely careful technic in order to secure specific and reliable results.

Precipitins do not permit of distinguishing different albumins of the same animal. For example, anti-ox precipitin prepared by injecting a rabbit with ox serum will react not only with ox-serum, but likewise with extracts of ox muscle, ox heart, ox kidney, ox pleural and pericardial fluids, etc. It would appear that every species of animal possesses throughout its tissues a common protein, but peculiar to its species. However, many organs un-

¹ Ztschr. f. Immunitätsf., 1920, 30, 351.

² Ztschr. f. Immunitätsf., 1921, 33, 348.

doubtedly possess peculiar proteins not present in other organs because by carefully freeing the organs from all blood and using extracts for immunization, it is possible to secure antisera that will yield precipitates best with the particular extract employed and a slight or no precipitate with extracts of other organs.

The most striking example of organ specificity is observed with the proteins of the lens of the eye. As shown by Uhlenhuth and since amply confirmed by Hektoen¹ and others, immunization of rabbits with this substance results in the production of a precipitin that does not react with the serum or any protein of the animal from which the lens was taken, but does react with the lens protein of the same animal and of all other animals in general. Hektoen² has recently described specific precipitins for erythrocytes—*erythroprecipitins*. Hektoen and Meune³ have also prepared specific *leukoprecipitins* for the leukocytes of the dog, guinea-pig, and human being. In other words, leukocytes appear to contain specific precipitinogenic substances not found in serum, platelets, or erythrocytes.

In medicolegal work, therefore, a diagnosis of "human blood stain" cannot be made without chemical evidence to prove that the stain actually consists of blood. For example, an extract of a stain of human albuminous urine may react with antihuman serum; or a stain of semen, blister fluid, pleural exudate, and according to Nuttall,⁴ even to a slight extent with nasal and lacrimal secretions (these may be avoided by using high dilutions of antigen or antiserum).

Besides this animal specificity, precipitin reactions also demonstrate the "constitutional specificity" of proteins. If, instead of using a pure animal or plant albumin for immunization, variously altered albumins are used (heated albumins, acid albumin, formaldehyd albumin, and the like), the organism reacts by producing antibodies of a characteristic nature, differing from those developed after inoculation with pure albumin. For example, if a rabbit is immunized with normal horse-serum, the resulting immune serum will produce a precipitate when added to pure horse-serum, but not when added to horse-serum that has been heated. On the other hand, if a rabbit is inoculated with horse-serum that has been diluted and boiled for a short time, the resulting immune serum will react not only with normal horse-serum but also with heated serum and a group of its decomposition products with which the normal immune serum ordinarily never produces a precipitate.

This observation is of practical importance in detecting meat substitution by precipitin reactions. In order to render the detection difficult, the meat is commonly boiled; with the aid of precipitins produced by immunization with heated proteins, this fraud is more easily detected than if a normal immune serum were used.

Obermeyer and Pick have demonstrated that while animal specificity is not destroyed when the albumins are modified by heat, tryptic digestion, or oxidation, their specificity is lost when an iodine, nitro- or diazo-group is inserted into the protein molecule. Immunization with such transformed proteins, *e. g.*, xanthoprotein, can produce a precipitating serum that will react with every xanthoprotein, even that of different animals. These investigators conclude that species specificity is probably dependent upon a certain aromatic group of the protein molecule.

¹ Jour. Amer. Med. Assoc., 1921, 77, 32.

² Jour. Infect. Dis., 1922, 31, 32.

³ Jour. Amer. Med. Assoc., 1922, 79, 1328.

⁴ Loc. cit., 105.

Rôle of Precipitins in Immunity.—Precipitins as they occur in the blood are probably not directly destructive for their antigens as are the anti-toxins and cytotoxins. Their rôle in resistance to infection and in the mechanism of recovery appear to be quite similar to the agglutinins. The latter, for example, do not kill or greatly injure the cells upon which they act, but yet agglutination greatly aids bacteriolysis or cytolysis in general, although these phenomena may occur without visible agglutination. The antigen may be recovered from a precipitate unaltered, as judged by its power of actively sensitizing guinea-pigs anaphylactically, just as living bacilli may be recovered from an agglutinated mass or found fully viable after being acted upon by specific opsonin. In other words, the precipitins appear to bear the same relation to dissolved albumins as agglutinins and opsonins do to formed elements, preparing or sensitizing the dissolved antigen for final destruction or albuminolysis. This subject is discussed more fully in the succeeding paragraph.

Relation of Precipitins to Albuminolysins; Complement Fixation by Precipitates.—In 1902 Gengou¹ showed that precipitates had the power of fixing or absorbing complement and concluded that immune sera prepared by injecting rabbits with milk, serum, egg-white, etc., contained not only precipitins but amboceptors or sensitizers as well, the latter being carried down in the precipitate and yielding the positive complement-fixation reactions. Gay,² however, who has studied this problem with particular care, came to the conclusion that complement was fixed by a process of absorption by the precipitate alone independent of the possible coexistence of sensitizers or amboceptors. The work of Zinsser³ has yielded a great deal more information upon this interesting and important subject. His experiments indicate that in immunization with formed antigens as bacteria, the immune serum contains bacterioprecipitin, and bacterial sensitizer or amboceptor, but that the two antibodies are identical, the precipitin being a sensitizer for dissolved antigen (albumins), and the amboceptor a sensitizer for the formed antigen (cells). When animals are immunized with dissolved antigen (albumins) as serum or milk, precipitins alone are produced, that is, sensitizers for the dissolved albumins. According to this view, therefore, the precipitins are to be regarded as protein sensitizers or amboceptors by which foreign proteins are rendered susceptible to the proteolytic action of alexin or complement. If this is correct, and I believe we may logically accept this view, complement fixation by precipitates is not one merely of mechanical absorption, but the fixation of complement by specific sensitizer which is the precipitin itself. Visible precipitation in such reactions is not necessary, and when it occurs is merely secondary because of the colloidal nature of the antigen and antibody and favorable quantitative and environmental conditions.

PRACTICAL APPLICATIONS

PHYTOPRECIPITINS

Bacterial Precipitinogens in the Urine in Pneumonia.—Dochez and Avery⁴ have shown that patients suffering from lobar pneumonia may excrete in their urine at some stage of the disease a soluble substance of pneumococcus origin. This substance gives a specific precipitin reaction with antipneumococcus serum corresponding in type to the organism with which

¹ Ann. d. l'Inst. Pasteur, 1902, 16, 734.

² Univ. Calif. Publ. Path., 1911, 2, 1; Jour. Immunology, 1916, 1, 83.

³ Jour. Exper. Med., 1912, 15, 529; *ibid.*, 1913, 18, 219.

⁴ Proc. Soc. Exper. Biol. and Med., 1916-17, 14, 126.

the individual is infected. A study of 111 cases of pneumonia has shown that in 65 per cent. of those due to pneumococcus Types I, II, and III, this precipitinogen is present in the urine, and can be detected by means of the appropriate antipneumococcic serum. The precipitinogen may appear in the urine as early as twelve hours after the initial chill, or it may appear for the first time at a later stage of the disease. It is a rule to find this substance when pneumococcus septicemia exists, being indicative of severe intoxication and an unfavorable prognosis. Quigley¹ has found this precipitin reaction in 67 of a series of 82 cases of Types I, II, and III pneumonia. The technic (described later) is simple, rapid, and accurate; while it should not supplant the mouse agglutination test for type diagnosis, I have found it of value as a confirmatory test.

Bacterial Precipitinogens in Inflammatory Exudates.—Probably the earliest application of the precipitin test for diagnosis with inflammatory exudates was by Vincent and Bellot² in the diagnosis of meningococcus meningitis. The spinal fluid is tested for meningococcus precipitinogen by overlaying with polyvalent antimeningococcus serum. Letulle and Legane,³ Bruynoghe,⁴ and Robinson⁵ have found this test of some value. Worster-Drought and Kennedy⁶ observed 12 fluids from 28 cases to react positively. The test may prove of diagnostic value when confirmatory evidence is required.

Schurman⁷ and Lacy and Hartman⁸ have found pneumococcus precipitinogen in the cerebrospinal fluid in pneumococcus meningitis and have advocated the precipitin test as an aid in diagnosis. The technic is described later.

Schurman, and later Floyd⁹ have also tested the pus from cases of pneumonic pleuritis and found a large percentage to contain specific precipitinogen corresponding to the type of pneumococcus and detectable by a precipitin test employing corresponding antipneumococcus sera.

Blake¹⁰ has found a precipitinogen in the peritoneal exudates of mice inoculated intraperitoneally with pneumonic sputum for the purpose of differentiating types of pneumococci by the agglutination test. The clear peritoneal fluid secured by centrifuging the exudate is tested with antipneumococcus sera of Types I, II, and III and the test has proved a valuable adjunct to the agglutination test.

Bacterial Precipitins in Anthrax, Bubonic Plague, Glanders, Tuberculosis, Gonorrhea, Echinococcus, Syphilis, and Other Diseases.—Wladimiroff¹¹ was apparently first to apply the precipitin test for the diagnosis of a bacterial infection, namely, glanders, finding a precipitin in the serum of a glandered horse for a filtrate of a forty-six-day-old culture of *Bacillus mallei* in glycerin-veal broth. Since then many investigators have described precipitin reactions in other bacterial diseases, and in some instances have advocated the test for diagnostic purposes, notably Ascoli¹² and Roncaglio¹³

¹ Jour. Infect. Dis., 1918, 23, 217.

² Bull. et mém. Soc. méd. d. hôp., 1909, 27, 952.

³ Compt. rend. Soc. de Biol., 1909, lxvi, 758.

⁴ Centralbl. f. Bakteriöl., 1911, 7, 38.

⁵ New York Medical Journal, 1919, cix, 464.

⁶ Cerebrospinal Fever, 1919, A. & C. Black, London, 272.

⁷ Med. Klinik, 1915, 11, 741.

⁸ Jour. Immunology, 1918, 3, 43.

⁹ Jour. Immunology, 1920, 5, 321.

¹⁰ Jour. Exper. Med., 1917, 26, 67.

¹¹ St. Petersburg Med. Wchn., 1900.

¹² Centralbl. f. Bakteriöl., 1911, 58, 63.

¹³ Ztschr. f. Immunitätsf., 1912, 12, 380.

for anthrax; Hecht¹ for symptomatic anthrax; Isabolinsky and Patzewitsch² for swine erysipelas; Müller³ for glanders; Berlin,⁴ Finzi,⁵ and Piras⁶ for bubonic plague; Watabiki⁷ for gonorrhea; Baldwin,⁸ Porter,⁹ Fuchs,¹⁰ Ferro,¹¹ Smeeton,¹² and others for tuberculosis; Welsh, Chapman, and Storey¹³ have employed the test for the diagnosis of echinococcus disease, reporting the positive reaction as possessing diagnostic value; Coroma¹⁴ has employed it in leishmaniasis. But these tests for diagnostic purposes are not nearly as satisfactory as the simpler agglutination test, and quantitative reactions are not elicited as readily and decisively as with the agglutination and complement-fixation tests, which have largely superseded the precipitin test. A possible exception is the precipitin reaction in the diagnosis of glanders by Ascoli's method. Reinhardt¹⁵ has recently renewed interest in this subject and a large number of investigators have reported favorably upon the test as a diagnostic reaction.

In *syphilis* Fornet and Schereschewsky¹⁶ have shown that in mixtures of syphilitic sera and sera from long-standing cases of syphilis, as paretics and tabetics, a precipitate may form. The serum to be tested is diluted five to ten times and placed in a small test-tube; undiluted parietic serum is overlaid and as it falls and mixes with the diluted serum sometimes produces a precipitate in two hours at room temperature. The reaction occurs infrequently and has no diagnostic value.

Bacterial Precipitins for the Identification and Differentiation of Bacteria.

—Noble has employed the precipitin test with rabbit immune sera for a study of the biologic relationship of members of the typhoid-colon group of bacilli and concluded in a comparative study with the agglutination reaction that the former yielded more delicate results and greater differentiation. The consensus of opinion, however, does not support this conclusion; personally, I have found the agglutination and especially the complement-fixation reactions far more sensitive and decisive in their results.

Yeast Precipitins.—Schütze has immunized rabbits with different yeasts and obtained precipitins as previously stated, but no one to my knowledge has employed the precipitin test for the practical diagnosis of yeast and fungous infections.

ZÖÖPRECIPITINS

Precipitins in the Examination and Identification of Bloods, Blood-stains, and Sera (the Hematoprecipitins).—Since the simultaneous discovery of the medicolegal use of the precipitin test by Uhlenhuth¹⁷ and Wassermann and Schütze¹⁸ a very large number of investigations have proved its practical value for the identification of blood-stains. The voluminous literature has been summarized by Nuttall,¹⁹ Uhlenhuth and Weidanz²⁰ together with reports of their own extensive investigations. In America Hektoen²¹ has

¹ Centralbl. f. Bakteriöl., 1913, 67, 371.

² Centralbl. f. Bakteriöl., 1913, 67, 284.

³ Ztschr. f. Immunitätsf., 1910, 8, 626.

⁴ Centralbl. f. Bakteriöl., 1915, 75, 467.

⁵ Centralbl. f. Bakteriöl., 1913, 68, 556.

⁶ Centralbl. f. Bakteriöl., 1913, 69, 69.

⁷ Jour. Infect. Dis., 1918, 22, 115.

⁸ Jour. Med. Research, 1904, 12, 235, 243.

⁹ Jour. Infect. Dis., 1910, 7, 87.

¹⁰ Blood Immunity and Blood Relationship, Cambridge University Press, 1904.

²⁰ Praktische Anleitung zur Ausführung des biologischen Enveissdifferenzierungsverfahrens mit besonderer Berücksichtigung der forensischen Blut und Fleischuntersuchung sowie der Gewinnung präzipitierender Sera, Gustav Fischer, Jena, 1909.

²¹ Jour. Amer. Med. Assoc., 1918, 70, 1273; Jour. Infect. Dis., 1922, 31, 32.

¹⁰ Centralbl. f. Bakteriöl., 1918, 81, 178.

¹¹ Riforma med., 1920, 36, 907.

¹² Amer. Rev. of Tuberculosis, 1922, 6, 588.

¹³ Australian Med. Gaz., December, 1908.

¹⁴ Ztschr. f. Immunitätsf., 1914, 20, 174.

¹⁵ Monatsch. f. Prak. Tierhl., 1920, 31, 268.

¹⁶ Berl. klin. Wchn., 1908, 18, 874.

¹⁷ Deutsch. med. Wchn., 1901, 27, 82.

¹⁸ Berl. klin. Wchn., 1901, 38, 187.

made numerous and valuable contributions to our knowledge of precipitins, especially methods for their production. He has also recently shown that precipitins prepared by immunization of rabbits with watery extracts of corpuscles are specific for the erythrocytic constituents, the hemoglobin acting as a species specific precipitinogen.

In Germany the test has become popular for the medicolegal identification of blood-stains, being very successful in the hands of Uhlenhuth. Stains on clothing, wood, metal implements, on plaster walls, in earth, on straw, and hay, etc., are readily detected and identified.

Contrary to popular opinion the test is not as simple as believed and in medicolegal work especially, requires potent antisera, experience, and a good working knowledge of the technic in order to secure reliable results. Group precipitin reactions must be scrupulously avoided and numerous controls included. The test, however, is more apt to err on the negative than on the positive side, a striking example being recorded by Hunt.¹ Personally I have found the complement-fixation test far more sensitive and satisfactory and have never consented to testify in medicolegal cases before conducting these tests in addition to the precipitin tests.

I have already discussed the specificity of the reaction earlier in this chapter; with proper attention to technical details the reaction is specific. Only the question of monkey blood can enter under these conditions, but in the United States this possibility can usually be excluded.

It is highly important to remember, however, *that stains of other fluids containing human albumins cannot be differentiated from blood-stains by precipitin tests*; microscopic or chemical tests must first establish that a stain is blood. With garments partly washed this is sometimes difficult. Furthermore, *it is not possible to distinguish between different human races nor between individuals by the precipitin test*. Therefore, in medicolegal cases involving the charge of murder these tests cannot determine whether a stain is due to the blood of the victim or the accused.

Precipitins in the Examination and Identification of Seminal Stains (Spermatoprecipitins).—In medicolegal cases involving the charge of rape these tests are occasionally of value. Immune sera are best prepared by injecting rabbits with human semen secured by prostatic and vesicular massage for therapeutic purposes.

Apparently the first specific antisemen precipitins were produced by Farnum² at the suggestion of Hektoen. Strube³ also produced them, but found these precipitins acted upon serum proteins as well, and he was not able to remove these by adsorption. Pfeiffer⁴ succeeded in producing a specific antisemen precipitin serum for bull spermatozoa by removing all precipitins for serum and organs other than the testicle by adsorption. Hektoen⁵ has recently confirmed this work and prepared specific antisemen sera, and believes that this precipitin reaction may prove of value in determining the nature of spots suspected to be of seminal nature. The technic of the tests is described later in this chapter.

Hektoen immunizes rabbits by giving four or five intramuscular injections of human semen at intervals of three or four days, beginning with 2 c.c. and increasing the quantity by 2 c.c. each succeeding injection. The animals are bled from six to eight days after the last injection.

¹ Boston Med. and Surg. Jour., 1917, clxxvi, 48.

² Jour. Amer. Med. Assoc., 1901, 37, 1721.

³ Deutsch. med. Wchn., 1902, 28, 425.

⁴ Wien. med. Wchn., 1905, 18, 637.

⁵ Jour. Amer. Med. Assoc., 1922, 78, 704.

Serum precipitins are removed from the rabbit immune serum by mixing equal parts of serum and a 1 : 200 dilution of human serum in saline. This mixture is left at room temperature for one hour in a refrigerator over night and thoroughly centrifuged. The supernatant fluid is employed for precipitin tests.

Precipitins in the Examination and Identification of Meats (Musculoprecipitins).—Uhlenhuth¹ has shown that the precipitin test may be utilized for the identification of different meats and especially for the detection of meat adulteration, as the presence of dog, cat, and horse flesh in sausages. Von Rigler,² Schmidt,³ and numerous others have confirmed these results; Gay⁴ has been able to identify the heart of a deer by this method and differentiate it from the heart of a calf, being the first to apply the musculoprecipitin test in a medicolegal case with conviction for an infringement of the game laws of Massachusetts.

Immune sera are prepared by injecting rabbits with extracts of flesh or with the corresponding sera.

The precipitins are not specific for the flesh extracts alone, that is, they will react almost as well with extracts of the different organs of the same animal or with the serum. In other words, when the meat for examination is minced, the precipitin test cannot differentiate between the minced muscle of the heart—say of the dog—but can identify the presence of the dog muscle.

Smoking of meat does not appear to interfere with the test; neither does salting, but cooking may. For the detection of cooked meat it is necessary to employ immune rabbit sera prepared by injecting sera diluted with saline and heated at 70° C. for thirty minutes or briefly boiled; very powerful antisera prepared by injecting unheated serum may suffice, this subject having been previously discussed under the coctoprecipitins.

The musculoprecipitins are apt to show the same species of relationship as the blood precipitins; for example, it is not possible to differentiate among the meats of the horse, mule, and donkey.

In practical work I have found the complement-fixation test much superior to the precipitin test for the identification and differentiation of meats; I have not been particularly successful with the musculoprecipitin reaction and prefer the former for the purpose of securing sharper and more conclusive results.

Precipitins in the Examination and Identification of Milks (Lactoprecipitins; Lactosera).—The investigations of Bordet,⁵ Neoro,⁶ Wassermann and Schütze,⁷ Hamburger,⁸ Bauereisen,⁹ Valerio and Bornand,¹⁰ and numerous other investigators have shown that precipitins obtained by injecting cow's milk into rabbits react with cow's milk, but not with human milk, and vice versa. Amberg¹¹ has shown that the precipitins secured by injecting whole milk are identical with those induced by the injection of casein.

The lactoprecipitins show the same species specificity as the correspond-

¹ Deutsch. med. Wchn., 1901, 27, 780; Ztschr. f. Immunitätsf., Ref., 1909-10, 1, 525.

² Oest. Chem. Zt., 1902, 5, 97.

³ Ztschr. f. Immunitätsf., 1912, 13, 166.

⁴ Jour. Med. Res., 1908, 19, 219.

⁵ Ann. d. l'Inst. Pasteur, 1899, 13, 225.

⁶ Wien. klin. Wchn., 1901, 1073.

⁷ Deutsch. med. Wchn., 1900, 178.

⁸ Wien. klin. Wchn., 1901, 1202.

⁹ Ztschr. f. Immunitätsf., 1911, 10, 306.

¹⁰ Ztschr. f. Immunitätsf., 1912, 14, 32.

¹¹ Jour. Med. Research, 1904, 12, 341.

ing seroprecipitins; for example, the precipitin for cow's milk will not precipitate human milk and vice versa; but cow precipitin reacts with the milk of the goat just as is true of the sera precipitins for these two animals.

Human seroprecipitin is likely to precipitate human milk, but lacto-sera are better prepared by immunizing rabbits with milk. Reactions are commonly observed with boiled milk (thermoprecipitinogen).

In my experience the complement-fixation test is very much superior to the precipitin test for the identification and differentiation of milks; the latter requires a large amount of immune serum for each test and the results have never been in my experience as clear cut and decisive as the complement-fixation reactions.

Precipitins for the Examination and Identification of Bones (Osteoprecipitins), Eggs (Ovoprecipitins), and Other Substances.—Beumer¹ and Schütze² have successfully identified bits of bone too small for recognition by inspection. Seroprecipitins were used with extracts of the bones, prepared as described in the section on Technic. Fine charred human bones have been identified, but I do not know the value of this method for the identification of decomposed bones; presumably the bone examined must contain some organic matter, as the reaction does not occur with the inorganic constituents.

Uhlenhuth³ and others have prepared antisera for egg-albumen by injecting rabbits with the white portions of hen eggs. These have been employed for the detection of egg-white in prepared foods.

Hen ovoprecipitin is likely to react with the albumins of the eggs of the pigeon and guinea-hen; in other words, their specificity is closely similar to the sero- or hematoprecipitins.

By means of these precipitins Grulee and Bonar⁴ have detected egg-albumen in the urine of infants; negative results were observed in a study of feces.⁵ Hektoen, Fantus, and Portis⁶ in a study of the precipitin reaction with feces found positive reactions with antihuman sera and extracts of the feces of healthy persons, indicating the presence of human proteins probably derived from the blood- and epithelial cells. Tests with anti-beef, antichickens, antisheep, and other sera with extracts of feces of healthy men on unrestricted, full meat diet were generally negative, indicating that in health foreign proteins taken in food do not reach the feces as such.

Berghausen,⁷ Hektoen, and Neymann⁸ have also employed the precipitin test for a study of the proteins in cerebrospinal fluid. The former reached the conclusion that the test was too delicate for clinical purposes; the latter believe that the test is adapted for routine use. Antisera were prepared by injecting animals with spinal fluid or the globulin and albumin fractions. With the fluids from paretic reactions were observed with dilutions 1 : 16 to 1 : 512, and higher similar results being observed with fluids from other cases of acute meningitis. Normal fluids did not react in dilutions higher than 1 : 3.7. Hektoen⁹ has also recently described a precipitin reaction for Bence-Jones albumin in urine.

It may be stated that specific organic reactions have been secured by

¹ Quoted by Nuttall, *Blood Immunity*, etc., 406.

² *Deutsch. med. Wchn.*, 1903, 29, 62.

³ *Deutsch. med. Wchn.*, 1900, 26, 734.

⁴ *Amer. Jour. Dis. Child.*, 1921, 21, 89.

⁵ *Amer. Jour. Dis. Child.*, 1920, 20, 15.

⁶ *Jour. Infect. Dis.*, 1919, 24, 482.

⁷ *Interstate Med. Jour.*, 1913, 20, 38.

⁸ *Jour. Amer. Med. Assoc.*, 1920, 75, 1332.

⁹ *Jour. Amer. Med. Assoc.*, 1921, 77, 929.



FIG. 109.—HEMIN CRYSTALS.

Prepared after the method described in the text. From a stain (over two months old) of sheep blood on a towel.

various investigators by prolonged immunization of rabbits with certain organ extracts. Thus it is possible to differentiate between the liver and kidney of the same animals; such tests have, however, but limited practical value. Maragliano attempted to apply this test of organic specificity to the serodiagnosis of malignant tumors by preparing immune serums by the injection of tumor juices, securing a serum that yielded a precipitate with the albumins of a cancerous tumor. The test is not absolutely specific, and its practical value in diagnosis requires confirmation.

Freund and Kaminer have described a precipitin reaction in cancer with an extract of cancer tissue, but the practical value of the test has not been established.

TECHNIC OF PRECIPITIN TESTS

DIFFERENTIATION OF HUMAN AND ANIMAL BLOOD—FORENSIC BLOOD TEST

Microscopic and Chemical Tests.—Unless a stain is definitely known to be a blood-stain it is *first necessary to establish its identity by making microscopic and chemical tests before proceeding with the precipitin reactions.* For example, old stains upon clothing may be due to substances other than blood, such as coffee and fruit juices; or, more importantly, they may be stains of some other human albuminous fluid which cannot be differentiated from old blood-stains. Blood-stains upon clothing, metal, wood, or glass may be used for making these reactions, and their source determined.

To identify the stain as one of blood a portion may be taken into solution in distilled water, rendered slightly acid with dilute acetic acid, filtered until clear, and examined spectroscopically. Or the *Teichmann hemin crystal test* may be applied to the stain by transferring to a clean slide a small amount of material scraped from the stain; add a few small crystals of sodium chlorid, crush the crystals, and mix the powder with the dry material. Place a clean cover-glass over the stained material and run a small amount of glacial acetic acid under the cover-glass. Heat the preparation to just about the boiling-point for a minute, replenishing the acid as may be necessary. The fluid turns brown. The specimen is allowed to cool a few minutes, and is then examined microscopically for the presence of brown rhombic crystals of hemin (Fig. 109). It may be necessary to reheat the specimen several times before the crystals are obtained. With stains in cloth and particularly those partially removed by washing, other chemical methods for detection, as the guaiac, benzidin, or Fürth¹ leukomalachite-green tests must be employed.

Kastle² has found solutions of phenolphthalein in alkali containing the quantity of hydrogen peroxid required for the oxidation of the phenolphthalein very satisfactory for the detection of blood. The reagent is prepared by dissolving 0.160 gm. of phenolphthalein in 105 c.c. of N/10 sodium hydroxid (prepared of pure product and accurately titrated), and diluting with redistilled water to nearly 500 c.c. To this solution is added 0.5 c.c. of M/1 hydrogen peroxid (0.5 c.c. of the 3 per cent. commercial product being sufficiently accurate), and the solution made up to exactly 500 c.c. with redistilled water. This solution keeps in glass-stoppered bottles in a dark place and may show only a faint pink color, which does not interfere with its use.

In conducting the tests 1 c.c. of the solution of substance containing blood is mixed with 2 c.c. of the reagent; a control is put up with 1 c.c.

¹ Fürth-Smith, *Physiological and Pathological Chemistry of Metabolism*, 1916, Lippincott & Co., 546.

² Hygienic Lab. Bulletin, 1909, No. 51.

of distilled water and 2 c.c. of the reagent. After five to fifteen minutes at room temperature a deep pink color indicates a positive reaction.

With this test Kastle was able to detect 1 part of blood to 8,000,000 parts of water; of course, the test is not as sensitive as this in the presence of urine, feces, gastric contents, pus, etc., but still possesses a high degree of sensitiveness and is worthy of trial for the detection of occult blood.

Having shown that a given stain is actually a blood-stain the source of the blood may be determined as the result of the precipitin reaction, which consists in extracting the stain in normal salt solution and mixing with antiserums prepared by immunizing rabbits with human and various animal serums. Since the antiserums are known, a precipitate with any one of the extracts indicates that the blood in the stain was derived from the same species of animal. The reaction is based upon the principle of the specificity of antigen and its antibody. Here the antibody is known, and is used in the test to detect the antigen.

As mentioned elsewhere, because of the presence of group precipitins the reaction is fraught with certain technical difficulties of importance, especially in medicolegal cases. In most instances it may suffice to show that a stain is of human blood as will be indicated by a strong reaction with human blood, and negative reactions with the bloods of lower animals. If the reaction is negative with antihuman serum the antiserums of the domestic animals, such as that of the dog, cat, hog, ox, horse, etc., are tried. Although the blood of the higher apes, and even of the lower orders of monkeys, may react slightly with human blood, this factor may be determined by observing a proper technic of dilution, or the possibility of a given stain being one of monkey blood definitely ruled out.

Preparation of the Extract of Stain (the Precipitinogen).—If the stain is on clothing or paper, a portion should be carefully removed and torn into shreds with forceps and scissors, and not with the fingers, and placed in normal salt solution. If the stain is upon wood, glass, or metal, the staining substance should be carefully scraped off with a *clean*, and preferably, sterile knife and placed in the salt solution. *As a further control on the technic an unstained portion of the clothing should be extracted in the same manner* in order to show that the latter alone does not give the reaction. The mixtures may be gently shaken, and should be stood aside for from two to twenty-four hours in a cold place to prevent bacterial multiplication, depending upon the rapidity of extraction. If the stain has been washed large portions of fabric must be extracted. Sometimes it is advisable to tease portions into single threads which may show incrustations, and especially if the cloth is of a heavier sort.

As a general rule, stains on fabrics and paper offer no difficulties, but small stains scraped from wood, metal, and leather surfaces may come away in small flinty flakes that dissolve very slowly in salt solution. With these it is sometimes advisable to extract with sterile distilled water diluting the final extract with an equal part of 1.8 per cent. saline to restore isotonicity. In the presence of insoluble bloods Uhlenhuth advises extracting with 0.1 to 1 per cent. solutions of soda. If these are employed it is necessary to carefully neutralize with decinormal hydrochloric acid using phenolphthalein for an indicator to avoid false precipitation. Ziemke recommends extracting with 1 per cent. solution of potassium cyanid, correcting the alkalinity by adding crystals of tartaric acid until the solution becomes neutral to litmus. I have sometimes aided extraction with saline solution by heating on a water-bath at 40° C. (not higher) for thirty minutes.

Small portions of blood-soaked plaster, earth, hay, straw, grass, etc.,

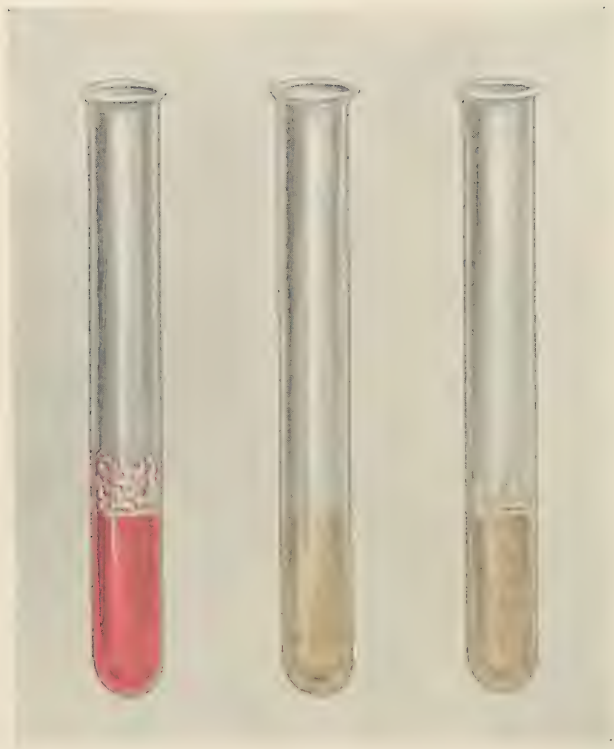


FIG. 110.—PRECIPITIN REACTION. PREPARATION OF EXTRACT OF BLOOD-STAIN.

The tube on the left shows the color of an extract of a blood-stain; the middle tube shows this extract so diluted as to yield a faint albumin reaction with nitric acid; the tube on the right shows the foam test with the same diluted extract (about 1 : 1000).

are extracted in the same manner as described for fabrics, saline solution being preferred for the solvent.

The reaction must be neutral to litmus. As a general rule, extracts are neutral to this indicator, but extracts of stains on *leather, wood, bark, and earth* may require neutralization with 0.1 per cent. solutions of sodium hydroxid or hydrochloric acid. As recently shown by Mason¹ specific precipitation will take place if the pH of solutions is 4.5 to 9.5 inclusive. However, if the pH is higher or lower than this range the precipitates may not form or are dissolved.

The extract should preferably not be stronger than 1 : 1000. The strength may be approximately estimated by the *foam test* by removing 1 c.c. of the extract into another tube and gently shaking or bubbling air through it. If a persistent froth appears upon the surface of the fluid sufficient extraction has occurred. Place 2 c.c. in a test-tube, heat to boiling, and add a drop of a 25 per cent. solution of nitric acid. A faint opalescence indicates that the strength of the extract is about 1 : 1000 (Fig. 110). If a heavy precipitate forms, the amount of normal salt solution that must be added to a portion of the extract to reduce it to a dilution of 1 : 1000 should be determined. The extract should be almost colorless by transmitted light, and must be crystal clear; this may be accomplished by filtering it through paper or a clean sterile Berkefeld filter. The filter shown in Fig. 50 is simple and very efficient.

When a blood solution is cloudy Nuttall drops some of it on a filter-paper, dries it for a half-hour or so in a thermostat, after which he places it in saline solution and thereby secures a clear solution without more ado.

In preparing an extract of whole blood the corpuscles should be laked with distilled water, the normal salt content being restored by adding an equal part of double strength saline solution (1.8 per cent.). Further dilutions are made with 0.9 per cent. saline solution.

When fowl antiserum is being employed, the antigen should be prepared by extracting with 1.8 per cent. saline solution, and the antiserum removed from a refrigerator should be allowed to stand at room temperature for an hour or two before use (Hektoen).

The Immune Serum.—Production.—A highly potent, sterile, and absolutely crystal-clear serum immune against the protein to be recognized must be prepared. For the recognition of blood-stains it is not necessary that whole blood be injected, as the serum alone will suffice.

Rabbits are generally employed; guinea-pigs produce precipitins very poorly. Sutherland² has observed that the fowl is well adapted for the preparation of precipitin sera; 5 c.c. of serum is injected into a wing vein, followed four days later by the injection of 10 c.c. Four days later 10 c.c. are injected intraperitoneally, the animal being bled two weeks later. Hektoen³ has found this method satisfactory; also that a single intraperitoneal injection of 20 c.c. of defibrinated blood or serum in most cases yields a precipitating serum in ten to twelve days of sufficient strength and specificity for practical purposes. Hektoen states that *these fowl antisera may yield non-specific reactions*, especially on rapid transfer from low to higher temperatures, and advises that 1.8 per cent. salt solution be used in making all mixtures and dilutions. Roosters should be selected instead of hens in order better to avoid opalescent sera.

Animals are injected with serum or blood (defibrinated or citrated);

¹ Johns Hopkins Hosp. Bull., 1922, 33, 116.

² Indian Jour. Med. Research, 1915, 3, 216.

³ Jour. Infect. Dis., 1918, 22, 561.

both are satisfactory, but serum is probably to be preferred, as the animals withstand better the effects of immunization.

Antihuman precipitins are especially difficult to prepare in sufficient potency for medicolegal tests; rabbits frequently succumb before their sera are ready. Fresh and preferably sterile sera should be employed. Smith¹ has described a useful method for preserving serum for purposes of immunization consisting of diluting 200 c.c. of defibrinated blood or serum with an equal volume of distilled water and adding 100 gm. of ammonium sulphate. The precipitate is collected next day by centrifuging, dried and ground into a fine powder which keeps indefinitely. When used, 0.5 gm. is suspended in 2 c.c. saline solution and injected intraperitoneally; this corresponds to approximately 10 c.c. of blood.

It is advisable to immunize several rabbits at the same time in case one or more succumb during the process of immunization. This is particularly true when human serum or blood are being employed.

Nuttall's Method.—Nuttall prefers the intravenous method, giving 3 to 5 c.c. serum every four to five days for at least four doses. After this time the animals should be tested periodically, and bled fourteen days after the last injection when the serum is satisfactory.

Uhlenhuth's Method.—Inject intravenously 2 or 3 c.c. of serum every five days for three doses, testing the serum seven days after the last injection, and daily for two or three days, bleeding in quantity when the trials show a valuable antiserum.

Hektoen's Method.—Inject 1 to 2 c.c. of serum intravenously and repeat after six days. Six days later 4 or 5 c.c. intraperitoneally, or from 5 to 6 c.c. of blood or serum may be injected intraperitoneally four or five times at intervals of six days.

The sera of the rabbits should be tested nine to twelve days after the last injection, and the animals bled while the precipitin content is high.

Rapid Methods.—Fornet and Miller,² Gay and Fitzgerald,³ and Hektoen⁴ have reported good results following the intraperitoneal injection of serum or defibrinated blood in doses of 5, 10, and 15 c.c. one day apart, bleeding about the twelfth day after the last injection.

Author's Methods.—The intravenous injection of 0.5 c.c. of defibrinated blood or serum every day for three weeks. A trial titration is made ten days after the last injection; if a serum is too feeble the animal receives six more daily injections.

This method has yielded good results; the mortality has been lower than with other methods, and the yield of acceptable sera quite good.

Or three intravenous injections may be given of 3 c.c. defibrinated blood or serum at intervals of three days followed by three intraperitoneal injections of 10 c.c. each at intervals of five days. The animals are tested ten days after the last injection; if a serum is too feeble the series of intraperitoneal injections is repeated.

Collection and Storage.—Most investigators agree that precipitin production reaches its maximum about ten to twelve days after the last injection of serum; as a general rule, this is the proper time for bleeding.

The sera of different animals should not be mixed, but kept separately in order to avoid clouding.

Collection and Storage of Sera.—The serum must be absolutely clear.

¹ Jour. Med. Research, 1916, 34, 169.

² Ztschr. f. Biol. Technik u. Methodik, 1908, 1, 201.

³ Univ. California Publ. in Path., 1912, 2, 77.

⁴ Jour. Infect. Dis., 1914, 14, 403.

Animals should be bled after a period of fasting, as the opalescence of the serum following feeding cannot be removed by filtration and will interfere with the reaction. Precipitin immune serum should be collected with a scrupulous aseptic technic, and stored in ampules holding 1 c.c. Although it is best not to add a preservative the addition of 0.1 c.c. of a 1 per cent. solution of phenol to each cubic centimeter of serum does not render the fluid cloudy and aids greatly in its preservation. Nuttall advises against the use of preservatives and prefers to remove bacteria by filtration if the serum has become contaminated.

If, after long standing, a precipitate has become deposited in an anti-serum, this should not be shaken up, but the ampule should be carefully opened and the clear supernatant serum drawn off with a capillary pipet. A serum that has become cloudy may be cleared partially or entirely by filtering it through a small candle filter (Fig. 50), although even an infected and offensive serum will give the reaction.

Apparatus.—Long and narrow test-tubes, 10 by 0.5 cm., are used. These must be absolutely clean, and preferably sterile.

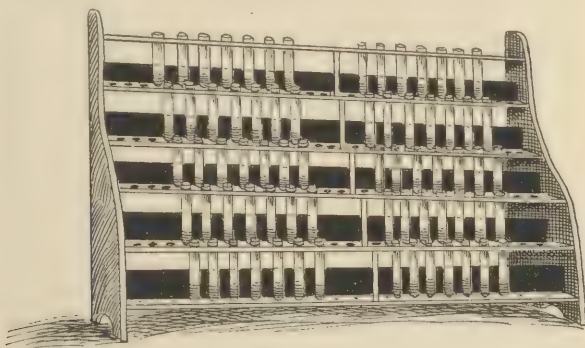


FIG. 111.—TEST-TUBE RACK FOR PRECIPITIN AND AGGLUTINATION REACTIONS. The strips of black material in the rear of the tubes facilitate reading the reactions.

The test-tube rack devised by Uhlenhuth in which the tubes hang suspended in beveled holes is quite satisfactory. Where a test is carried out with many controls, a rack similar to the one shown in the illustration (Fig. 111) is quite serviceable. A strip of black material placed behind the tubes aids greatly in the detection of the finer degrees of opalescence or precipitation.

Preliminary Titrations.—The precipitin content of an immune serum is titrated frequently during the process of immunization and after the animal has been bled. For medicolegal purposes Uhlenhuth advises the use of only highly valent serums. He considers an antiserum efficient if 0.1 c.c. of it, when added to its respective serum-antigen diluted 1 : 1000, produces a distinct turbidity, either at once or in from one to five minutes at the latest.

Into a series of six test-tubes place 1.0 c.c. of the following dilutions of serum-antigen, prepared with normal salt solution: 1 : 100, 1 : 500, 1 : 1000, 1 : 2000, 1 : 4000, and 1 : 8000. To each tube add 0.1 c.c. of clear immune serum. *The line of contact between serum and antigen should be sharp*; the serum may be very carefully run down the side of each tube to collect at the bottom, but it is better to introduce the pipet to the bottom of each tube. The tubes must not be shaken. Within from one to five minutes

a faint, misty cloud appears at the bottom of the tubes reacting positively, and this becomes a distinct precipitate within one-half to one hour (Fig. 112).

As previously stated, the immune serum should be of such strength as to produce a reaction within an hour with a 1 : 1000 dilution of antigen. Weaker sera react more slowly and may require as long as twenty-four

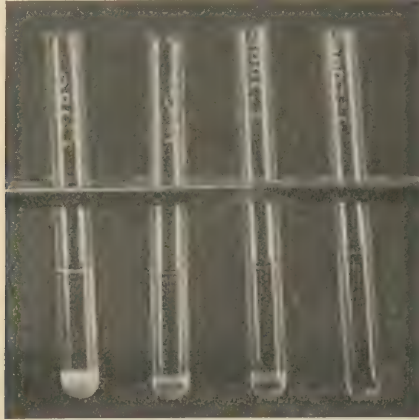


FIG. 112.—TITRATION OF A PRECIPITIN (SERUM).

Not all tubes of the series are here shown. Note the well-marked precipitate in the bottom of the first two tubes (1 : 100 and 1 : 500); the third tube (1 : 1000) shows less precipitate; the fourth tube (1 : 2000) is negative (clear and no precipitate). The titer of this serum was recorded as 1 : 1000.

hours; these are apt to be unsatisfactory. More powerful sera may react at once, and Uhlenhuth has cautioned against their use in medicolegal tests because of the increased danger of reacting with non-related bloods.

If more prolonged incubation is required it is better to incubate at 55° C. than at 37° C. to prevent bacterial contamination. I have observed that

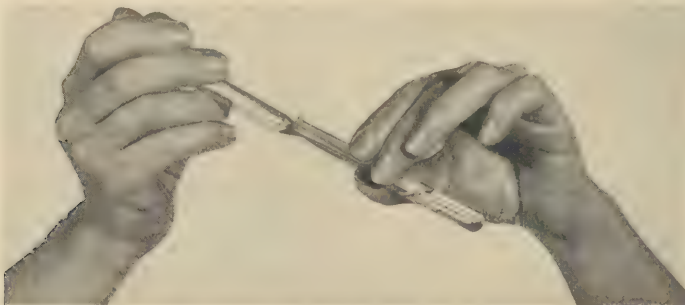


FIG. 113.—METHOD OF PLACING IMMUNE SERUM IN BOTTOM OF TEST-TUBE BY MEANS OF A PIPET TO SECURE A SHARP LINE OF CONTACT IN PRECIPITIN TESTS.

this intensifies a weak or indefinite reaction and does not materially reduce the activity of the precipitin or coagulate the precipitum.

Before performing the actual test with the unknown blood-stain it is advisable to test the entire reaction with a similar known blood-stain in order to make sure that all ingredients are in good working order. In laboratories equipped for medicolegal examinations stains upon filter-paper or

linen, of the blood of man, dog, cat, ox, horse, etc., and their respective antisera are always kept in readiness for making the preliminary and actual tests.

Technic of the Test.—The following mixtures are set up in a series of test-tubes. Fresh sterile pipets should be used in handling the various solutions. The immune serum should be added slowly, and in such a way that it will collect at the bottom with a sharp line of demarcation; for this purpose it is better to introduce the pipet to the bottom of each tube rather than flowing the serum down the sides (Fig. 113).

Tube 1: 1 c.c. of extract of unknown substance in dilution of 1 : 1000 + 0.1 c.c. of immune serum.

Tube 2: 1 c.c. of the unknown extract in dilution of 1 : 1000 + 0.1 c.c. of normal rabbit serum.

Tube 3: 1 c.c. of an extract of bloodless part + 0.1 c.c. of immune serum.

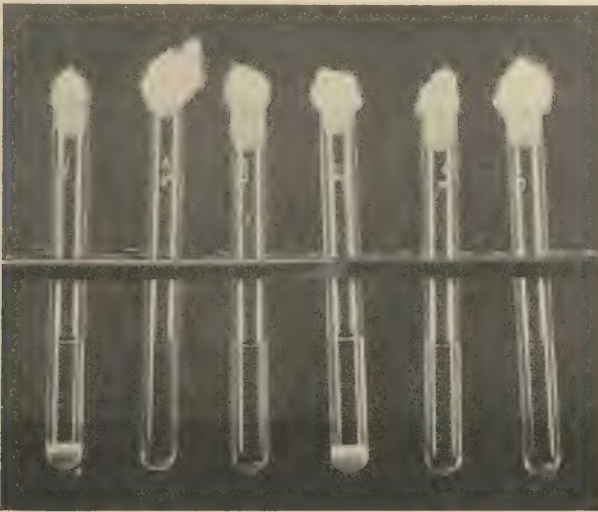


FIG. 114.—A PRECIPITIN REACTION. BIOLOGIC BLOOD REACTION.

Tube No. 1 contains an extract of blood-stain and antihuman serum (positive reaction). No. 2 is same with normal serum (negative). No. 4 is 1 : 1000 human serum and antiserum (positive control). No. 5 is extract of stain and saline solution (negative). No. 6 is antiserum and saline solution (negative). No. 7 is extract of sheep blood and antiserum (negative).

Tube 4: 1 c.c. of a 1 : 1000 dilution of the serum of that species of animal whose blood is suspected to be present in the unknown solution + 0.1 c.c. of immune serum (control).

Tube 5: 1 c.c. of the extract of unknown substance + 0.1 c.c. salt solution (control).

Tube 6: 0.1 c.c. of the immune serum + 1 c.c. of normal salt solution (control).

Tube 7: 1 c.c. of the extract of the blood of some other animal + 0.1 c.c. of immune serum (control).

The tubes are not shaken, are kept at room temperature, and the results are read after from ten to twenty minutes. Exposure to incubator temperature facilitates the reaction. With proper immune serums, and especially in medicolegal work, a positive reaction should appear within two to five minutes as a faint, misty cloud at the bottom of the test-tube.

Within five minutes this becomes more definite, and in from ten to twenty minutes the precipitate is seen (Fig. 114). Any cloudiness that develops later than twenty minutes after the beginning of the reaction has no significance.

In tests other than those employed in medicolegal work, especially if the antisera are weaker than desired, the reaction may be read after one to two hours.

In the foregoing test, if positive results are obtained in tubes 1 and 4, and all the others react negatively the presence of the blood or protein of the species suspected in the unknown extract is established. If the entire test proves negative, the species to which the unknown specimen belongs must be determined with new antisera prepared for each species, and the tests conducted in the manner described.

Partial reactions between closely related species due to group precipitins seldom occur, and are easily detected when the technic described is employed. The precipitin test, as determined by the extensive experience of Nuttall is highly specific, and it is only between very closely related animals, such as the hare and the rabbit, the horse and the mule, the sheep and the goat, etc., that any doubt can arise.

Sources of Error.—(a) *Opalescent Antisera.*—Uhlenhuth has sounded a warning against their use in medicolegal tests. A slight opalescence is usually perceptible when any serum or antiserum is added to blood dilutions, the tube being viewed by strong transmitted light, but the clouding here referred to is much more marked and takes place even in salt solution.

(b) Weak antisera which may yield falsely negative reactions or require prolonged incubation with consequent bacterial contamination. I have secured satisfactory reactions, however, with weaker sera than advised above by incubating at 55° C. for twelve to eighteen hours instead of at 38° C. This higher temperature prevents bacterial multiplication and does not interfere with the precipitin.

(c) Too powerful antiserum which may react with antigens of non-related bloods. With 1 : 1000 dilutions of antigen, however, there is little danger and particularly with the average antihuman rabbit serum.

(d) Too dilute extract of serum, blood, or other unknown substance being tested which leads to falsely negative reactions.

(e) Too concentrated extracts of antigen which may dissolve the precipitate or lead to non-specific group reactions.

(f) Too much preservative in the antiserum which may result in slight clouding.

(g) Too acid or too alkaline reaction of the antigen; particularly important with extracts of stains on leather, plaster, and in earth.

(h) Bacterial contamination which leads to clouding and collection of sediment. A source of danger only when the tests are incubated or left in a warm room for twelve to twenty-four hours.

Capillary Tube Method.—When only very limited amounts of the unknown stain are available, the test, according to Hauser, can be carried out in slender, clean, and sterile capillary tubes. The piece of stained clothing is torn into shreds, extracted, and filtered until clear. The tests are performed by drawing a small amount of the unknown solution into a capillary tube, and underlying this with a small amount of immune serum. As many controls as possible are put up in the same manner. A distinct whitish ring will form in the positive tubes at the line of contact between the two fluids; this is best seen by holding the tube against a black background.

DETECTION OF MEAT ADULTERATION

The principle of this method is the same as that in the foregoing test. An extract of a meat will yield a precipitate when mixed with its antiserum, prepared by immunizing rabbits with an extract of the flesh or with the blood-serum of some other animal.

The method is especially serviceable in food inspection for the detection of horse, dog, or other foreign flesh in meat mixtures, such as sausage and the like. Even salted and cooked meats may be used in the test, although the latter may require the use of antisera prepared by immunizing with heated or cooked antigen.

Preparation of the Meat Extract.—To prepare this about 50 gm. of flesh are removed from the deeper parts of the specimen by means of a sterile knife, and through a fresh opening, as this portion has been least exposed to the methods of preservation, especially at the high temperatures to which the meat may have been exposed. It should contain as little fat as possible. It is then placed on a clean sterile tile and cut into smaller pieces, and finally minced by passing it through a *perfectly clean* meat-grinder or chopping it with a sterile chopping knife. After being finely minced the meat is placed in a sterile Erlenmeyer flask, and covered with 100 c.c. of sterile normal salt solution. The mixture of meat and salt solution is kept for about six hours at room temperature, or overnight in the refrigerator, the flask being gently shaken from time to time.

Salted meat should be ground and freshened by placing it in a large sterile Erlenmeyer flask and covering it with sterile distilled water, renewed several times in the course of fifteen minutes without shaking the flask.

Graham-Smith and Sanger¹ have found that with extracts containing more than 5 per cent. sodium chlorid the antiserum did not sink to the bottom, and that clouding occurred at the top of the tubes and took longer in forming.

Since the presence of a great deal of fat interferes with the reaction, it is advisable to remove it beforehand by extracting it with ether and chloroform for from twelve to twenty-four hours (Miessner and Herbst). Pork sausages are usually quite fatty, and may require this preliminary treatment. To make the extraction, take about 75 to 100 gm. of the minced meat or sausage and place it in a large Erlenmeyer flask and cover with equal parts of ether and chloroform. After twenty hours the ether and chloroform are poured off, the meat is washed once or twice with sterile normal salt solution, and then extracted in 100 c.c. of salt solution as described above.

To determine whether a sufficient quantity of protein substances has passed into solution place 2 c.c. in a test-tube and shake vigorously. If a foam develops and persists for some time the extraction may be said to be complete. It must then be filtered until it becomes perfectly clear. With extracts of fresh lean meat this is usually accomplished by filtering through a hard filter-paper moistened with salt solution. If it is not crystal clear, and especially if the meat to be examined is fat or salt, it may be necessary to filter through a sterile Berkefeld filter.

To make the test the extract should contain about 1 part of protein in 500 parts of salt solution. To determine this, 2 c.c. of the clear filtrate are placed in a test-tube and heated, a drop of dilute nitric acid being added. If a marked cloudiness and a flocculent precipitate develops, the extract is too concentrated and must be diluted with clear normal salt solution

¹ Jour. Hyg., 1903, 3, 258.

until the heat and acid test causes only a diffuse, opalescent cloudiness that settles at the bottom of the tube after five minutes as a slight precipitate.

Before proceeding with the experiment the reaction of the solution should be tested with litmus-paper, and if it is found to be acid, it should be neutralized very carefully with $n/10$ sodium hydroxide.

Extracts of the meats that are known or likely to be present, such as extracts of pork and beef, should be prepared as controls.

Preparation of Immune Serum.—An immune serum against that variety of flesh that is to be determined in the unknown specimen is prepared by injecting rabbits intravenously with the serum of an animal of that species. For example, if the object is to test for dog meat, an antidog serum is prepared by immunizing rabbits with sterile dog serum.

As has repeatedly been mentioned it is advisable to immunize a number of rabbits at the same time, for only a small number will yield a satisfactory serum after the third injection.

Immunization may be performed with extracts of flesh that have been filtered and heated at 56° C. for an hour to secure partial sterilization. Such injections when given subcutaneously are likely to produce extensive sloughing, and with any method of immunization the mortality is high.

After the third inoculation it is well to remove a small amount of blood from the ear and make a preliminary titration. This is performed in exactly the same manner as in making the forensic blood test previously described. An antiserum is satisfactory if 0.2 c.c. produces a well-marked cloudiness, and a precipitate in ten minutes with 1 c.c. of a 1 : 500 or 1 : 1000 dilution of the serum or extract of flesh.

In addition to being highly potent the immune serum must be crystal clear and sterile. To avoid opalescence the animal should be bled only after a period of fasting.

When testing for cooked meats the antiserum should be prepared by immunizing rabbits with serum diluted with saline and heated at 70° C. for thirty minutes; antisera prepared by immunization with unheated sera may suffice, but better results are likely to occur with heated sera (see previous discussion on coctoprecipitins).

Technic.—If, for example, the object is to determine whether a piece of meat is horse flesh, or if sausage contains the meat of this animal, the test is conducted as follows:

Tube 1: 1 c.c. of unknown extract, 1 : 500 + 0.2 c.c. of antihorse-serum.

Tube 2: 1 c.c. of unknown extract, 1 : 1000 + 0.2 c.c. of antihorse-serum.

Tube 3: 1 c.c. of unknown extract, 1 : 500 + 0.2 c.c. of normal rabbit-serum.

Tube 4: 1 c.c. of horse flesh extract or horse-serum, 1 : 1000 + 0.2 c.c. of antihorse-serum (positive control).

Tube 5: 1 c.c. of serum of some other animal, 1 : 500 + 0.2 c.c. of antihorse-serum (control).

Tube 6: 1 c.c. of unknown extract + 0.2 c.c. saline solution (control).

Tube 7: 1 c.c. of sterile salt solution + 0.2 c.c. of antihorse-serum (control).

The immune serum is added to each tube very carefully and preferably by placing the pipet in the bottom of each tube or by flowing the serum down the sides. The tubes should not be shaken.

If the preliminary titration of the immune serum fulfils the ideal requirement of yielding a well-marked cloudiness within five or ten minutes with a 1 : 1000 extract, the foregoing test should be recorded at the end of half an hour at room temperature. If in tubes 1, 2, and 4 a misty cloudi-

ness should appear within five minutes, the extract is very probably one of horse flesh. If a definite precipitate forms within thirty minutes, the other tubes remaining clear, horse flesh or the flesh of some other single-toed animal is present.

If the preliminary titration does not show a precipitate with the immune serum until at the end of one or two hours, this interval may be utilized for conducting the test.

In a similar manner tests may be made for the meat of dogs, cats, or any other animals if the respective immune serums are used with the extract.

BACTERIAL PRECIPITIN TESTS

Bacterial precipitinogens are prepared by filtering ten to twenty-one day bouillon cultures through Berkefeld filters. The filtrates must be absolutely clear and sterile for the reaction frequently requires a number of hours, and if bacteria are present they may grow quickly, produce turbidity, and mask a reaction.

Ascoli prepares precipitinogens by washing off twenty-four-hour agar cultures with 5 to 10 c.c. saline solution, shaking for two hours and filtering through asbestos until clear.

If broth cultures are employed it is important that the reaction is not too acid or too alkaline in order to avoid flocculation of serum proteins; Noble¹ has found that broth + 1.5 to phenolphthalein produces slight flocculation. The reaction should not range more than -0.5 to $+1.0$.

Thermoprecipitinogens (Methods of Ascoli, Krumwiede, and Noble).—Extracts of bacteria in tissues are prepared by Ascoli (for anthrax) by boiling 1 gm. of minced spleen in 5 c.c. saline solution and filtering. Krumwiede and Noble² have described the following method for preparing precipitinogens of bacteria which, briefly, consists of growing the bacteria on large areas of agar; collecting and suspending them in distilled water; dissolving them by the addition of alkaline hypochlorite solution (antiformin), and boiling to clearness; neutralization with hydrochloric acid; precipitation with alcohol and extraction of the sediment with 0.8 per cent. salt solution at 100° C. The final extract is clarified by centrifuging.

Immune Serum.—This is prepared according to the technic described under Active Immunization. Rabbits are given intravenous injections of increasing doses of cultures of the bacteria themselves or of filtrates, the inoculum being heated at 60° C. for an hour previous to making the injection. After the third dose the serum is titrated and the injections continued unless the serum is satisfactory.

Technic.—A known quantity of serum and varying amounts of precipitinogen are employed. If too much precipitinogen is furnished, the precipitate will not form, and one that has already formed may dissolve on the addition of more precipitinogen.

If, for example, one desires to determine if anthrax precipitin is present in a given serum, the test is conducted as follows:

Tube 1: 0.5 c.c. antigen + 0.5 c.c. unknown serum very carefully and slowly run down the side of the tube in order to gather under the antigen with a sharp line of contact.

Tube 2: 0.5 c.c. of 1 : 10 antigen + 0.5 c.c. unknown serum.

Tube 3: 0.5 c.c. of 1 : 100 antigen + 0.5 c.c. unknown serum.

Tube 4: 0.5 c.c. of antigen + 0.5 c.c. known positive serum (positive control).

¹ Jour. Infect. Dis., 1904, 1, 463.

² Jour. Immunology, 1918, 3, 1.

The tubes are not shaken, and are kept at room temperature. If the unknown serum contains considerable anthrax precipitins, a positive reaction will be noticed in the first three tubes in a short time—often within from ten to fifteen minutes. Tube 4 should show a strong reaction and the other tubes should remain clear.

Quantitative Precipitin Test.—In studying the biologic relationship of an organism to others of the same group its immune serum may be used in amounts of 0.5 c.c. of varying dilutions with a constant dose of 0.5 c.c. of the bouillon filtrates of the various organisms studied. A comparison of the precipitates in the respective dilutions of the different filtrates indicates the relationship according to the amount of group precipitins present in the immune serum. The test may be conducted as follows:

Tube 1: 0.5 c.c. undiluted serum + 0.5 c.c. antigen very carefully overlaid.

Tube 2: 0.5 c.c. serum 1 : 2 + 0.5 c.c. antigen.

Tube 3: 0.5 c.c. serum 1 : 4 + 0.5 c.c. antigen.

Tube 4: 0.5 c.c. serum 1 : 8 + 0.5 c.c. antigen.

Tube 5: 0.5 c.c. serum 1 : 16 + 0.5 c.c. antigen.

Tube 6: 0.5 c.c. serum 1 : 32 + 0.5 c.c. antigen.

Tube 7: 0.5 c.c. serum 1 : 64 + 0.5 c.c. antigen.

Tube 8: 0.5 c.c. serum 1 : 128 + 0.5 c.c. antigen.

Tube 9: 0.5 c.c. undiluted serum + 0.5 c.c. saline (control).

Tube 10: 0.5 c.c. saline solution + 0.5 c.c. antigen (control).

The tubes are not mixed but handled very gently and allowed to stand at room temperature for at least one hour, the first reading being made after fifteen minutes.

Higher dilutions of immune serum may be employed.

The immune sera are prepared by injecting rabbits with the respective micro-organisms.

Absorption of Bacterioprecipitins.—Krumwiede and Cooper¹ have described a method for removal of group precipitins from immune serum by absorption with precipitinogens or the bacteria themselves. They have found absorption of only limited application in the differentiation of closely related bacteria with a tendency toward non-specific results, that is, absorption may remove not only the main precipitin, but the group precipitins as well.

Dochez and Avery's Precipitin Reaction with the Urine in Pneumonia.—The urine should be fresh and cleared by centrifuging. In each of a series of four small test-tubes place 0.5 c.c. urine with 0.5 c.c. *clear* anti-pneumococcus sera of Types I, II, and III; the fourth tube receives 0.5 c.c. saline solution and is a control. Mix the contents of each tube and place them in a water-bath at 37° C. for one hour. It is essential that the urine and sera be water clear. A positive reaction is indicated by a cloudy to heavy flocculent precipitate.

In case the reaction is negative or indecisive the urine may be concentrated by adding a few drops of acetic acid to 25 c.c. and boiling down to 5 c.c. followed by paper filtration. To this are added 50 c.c. of 95 per cent. alcohol and the precipitate collected by centrifuging. The precipitate is rapidly dried in an incubator and extracted with 3 c.c. of salt solution. This extract is cleared by centrifuging and employed as above.

Blake's Precipitin Reaction with Mouse Peritoneal Exudate in Pneumonia.—Mice are inoculated with sputum and the peritoneal exudate recovered as described for the agglutination test. After thoroughly centrif-

¹ Jour. Immunology, 1920, 5, 547.

using, the clear supernatant fluid is pipeted off with care not to disturb the sediment, and is used as follows:

- I. (1 : 10) + 0.5 c.c. peritoneal fluid.
- II. (undiluted) + 0.5 c.c. peritoneal fluid.
- II. (1 : 10) + 0.5 c.c. peritoneal fluid.
- III. (1 : 5) + 0.5 c.c. peritoneal fluid.

Incubation is usually not necessary. A positive reaction is shown by the development of a whitish ring at the line of contact or a diffuse cloudiness. A negative reaction in all tubes indicates pneumococcus belonging to Type IV. A positive reaction in Tube 2 and a negative reaction in Tube 3 indicates atypical pneumococcus Type II; a positive reaction in both Tubes 2 and 3 indicates typical pneumococcus Type II.

Oliver's Precipitin Reaction with Sputum in Pneumonia.—Oliver¹ has described the following method for the rapid and direct diagnosis of pneumonia: "After a direct smear of the sputum has been made from 1 to 2 c.c. are placed in a clean centrifuge tube. From 3 to 5 drops of undiluted ox bile (or a 10 per cent. solution of sodium taurocholate) are added and a sufficient quantity of sterile physiologic sodium chlorid solution, if necessary, to insure a specimen of sufficient fluidity to allow of centrifugation. The mixture is then thoroughly stirred and broken up with a glass rod; grinding in a small mortar with a pestle may be necessary. The tube is then heated in a water-bath at 42° to 45° C. for twenty minutes which suffices for a solution of the pneumococci by the bile. The fluid is then centrifugalized. Of the centrifugate, from 0.3 to 0.5 c.c. quantities are carefully pipeted into each of three small, scrupulously clean tubes. To the first tube is added from 1 to 2 drops of undiluted Type I pneumococcus serum, and to the second and third tubes the same quantity of Type II and Type III sera, respectively. A positive reaction is shown by an almost immediate clouding and flocculation which is enhanced by heating at 42° C. in a water-bath for from ten to twenty minutes."

Precipitin Reactions with Spinal Fluid in Meningitis.—The spinal fluid is centrifuged and the *clear* superfluid employed.

If pneumococcus meningitis is suspected the tests are conducted by placing 0.5 c.c. of fluid in each of three small test-tubes, adding 0.5 c.c. of Types I, II, and III antipneumococcus sera, respectively. These are mixed and incubated in a water-bath at 37° C. for one hour. Positive reactions are indicated by clouding and flocculation.

If meningococcus meningitis is suspected the test is conducted in the same manner with monovalent sera for the identification of type of meningococcus, or with a polyvalent serum.

Precipitin Reactions in Other Bacterial Infections.—Precipitin reactions have also been employed by Robinson and Meader² for the diagnosis of *gonococcus infections*, but in the experience of Kelley³ and others the reaction has not proved of practical diagnostic value.

Smith and Kaufman⁴ have recently described a precipitin reaction occurring with antigens prepared of swabs containing *diphtheria* bacilli from exudates prepared by a method described by the authors, and horse- or rabbit- immune sera. Of 74 cases controlled by cultural methods the precipitin reaction was found to yield satisfactory results.

Other Uses of the Precipitin Test. The Identification of Bone.—Most

¹ Jour. Infect. Dis., 1920, 27, 310; *ibid.*, 1921, 29, 518.

² Jour. Urology, 1920, 4, 551.

³ Jour. Infect. Dis., 1922, 30, 623.

⁴ Jour. Lab. and Clin. Med., 1922, 7, 619.

difficulty is encountered in the preparation of an extract. Soft tissues should be removed. The bone should be reduced to a powder; I have found sawing and collection of the dust most convenient unless small fragments may be pounded to dust with a clean hammer. Scrupulous cleanliness must be observed throughout to guard against accidental mixtures of proteins of other origin. It is better to handle the bone with forceps. The dust is extracted with salt solution as described for the preparation of extracts of meats. The extract should give a positive foam reaction, and be clarified if necessary by filtration.

Antiserum is prepared by injecting serum into rabbits. Usually the information sought is whether or not a fragment is human bone. The tests are conducted with an antihuman rabbit-serum exactly as described for the forensic blood test.

For the Identification of Milk.—Antiserum is prepared by injecting milk intravenously into rabbits in the same amounts advised for serum. I generally heat the milk at 60° C. for one hour to reduce the bacterial content before injection. The test may be conducted with antisera prepared by injecting rabbits with serum instead of milk.

In conducting the tests the milk antigen is prepared by diluting 1 : 25 with saline solution and filtering. To 1 c.c. of this antigen is added 1 c.c. of immune serum, followed by gentle mixing; the antigen may be stratified over 0.5 c.c. of the serum, but usually the ring cannot be clearly seen. A positive reaction is indicated by flocculation and collection of a precipitate.

Bordet conducted the test by adding 6 to 15 drops of milk to 3 c.c. of immune serum.

For the Identification of Semen.—These are usually stains on clothing. The antigen is prepared by extracting with physiologic saline solution for several hours. The extract should be concentrated by using minimal amounts of saline and a portion centrifuged. The sediment should be examined for spermatozoa; the heads resist deterioration for much longer periods than the tails. The balance of the extract may then be diluted until it gives a satisfactory foam reaction and a slight cloud when boiled and a few drops of acetic acid added. The extracts usually require clearing by filtration.

The antiserum may be prepared by injecting rabbits with human serum, semen, or testicular extracts. Immunization with semen yields the best serum.

The tests are conducted in exactly the same manner as the blood tests. Control extracts of guinea-pig testicle or other animal should be included.

As a general rule the immune sera also give precipitates with the serum of the animal corresponding to the species of spermatozoa employed for immunization. Pfeiffer,¹ however, secured specific antisemen sera by absorbing these group serum and organ precipitins. He injected rabbits with dried and powdered bull spermatozoa, suspended in salt solution; the resulting antiserum acted strongly on semen solutions and testicular extracts, and only feebly or not at all on extracts of other beef organs, and by treatment of the antiserum with beef serum and certain organ extracts all precipitins except those specific for semen could be removed. This treated antiserum caused precipitates in dilutions of bull semen, and detected bull semen in mixtures with organ extracts.

Hektoen² has recently confirmed this work for human semen. He prepared immune sera by injecting rabbits with semen secured in the usual clinical manner by massage of the seminal vesicles, as follows: four or five

¹ Wien. med. Wchn., 1905, 18, 637.

² Jour. Amer. Med. Assoc., 1922, 78, 704.

injections were made intramuscularly in rabbits at intervals of three or four days, beginning with 2 c.c. and increasing the quantity by 2 c.c. each succeeding injection. As a rule the best time to bleed the rabbits for serum was found to be from six to eight days after the last injection. As was expected, the immune sera gave precipitates with human serum as well as with human semen. To remove the precipitin for human serum equal parts of antiserum and 1 : 200 dilution of human serum in normal saline solution were mixed, left at room temperature for about one hour and in the ice-box overnight, and then thoroughly centrifuged. As a rule this procedure removes all precipitin for human serum and leaves a serum specific for human semen, as shown in the following table taken from Hektoen's paper:

SPECIFIC PRECIPITINS FOR HUMAN SEMINAL PROTEINS IN SERUM OF RABBITS INJECTED WITH HUMAN SEMEN

SERUM OF RABBITS INJECTED WITH HUMAN SEMEN.	TITERS OF ANTISERUM IN			
	Human Serum.	Human Seminal Fluid.	Animal Seminal Fluids (Bull, Boar, Dog, Guinea-pig, Rabbit, Rat).	Salt Solution.
1. Original.....	6400	800	0	0
Treated.....	0	256	0	0
2. Original.....	3200	256	0	0
Treated.....	0	64	0	0
3. Original.....	6400	640	0	0
Treated.....	0	256	0	0
4. Original.....	6400	640	0	0
Treated.....	0	320	0	0
Normal rabbit serum.....	0	0	0	0

The figures give the highest dilution of serum and seminal fluid in which the antiserum caused distinct precipitates by the layer or contact method after one hour at room temperature.

The clear fluid secured by centrifuging semen or salt solution extracts of stains is employed as antigen; the former should be diluted about 1 : 100. The tests are conducted by placing 1 c.c. of seminal solutions in test-tubes and introducing in the bottom of each 0.1 c.c. of immune serum by means of a pipet in order to secure sharp contact. The readings are made after one hour at room temperature.

CHAPTER XVIII

CYTOLYSINS

Amboceptors (Sensitizers) and Complements (Alexins)

General Considerations.—The *cytolysins* include a number of antibodies of considerable diagnostic and therapeutic importance, for example, the hemolysins and the bacteriolysins. It will be remembered that the various antibodies act differently upon their antigens, and that, according to the side-chain theory, as their antigens become more highly organized, their structure becomes more complicated. For example, the molecule of a soluble toxin may be considered as simple in structure, and accordingly its antibody has been conceived as being likewise simple, and composed of a plain cast-off receptor or side-arm that unites directly with the toxin and neutralizes it without further aid. Antitoxins and antiferments are antibodies of this nature. For more highly organized antigens, however, so simple an antibody will not suffice, and we now find a more complicated antibody, composed of a portion that unites with the antigen and another portion, an integral part of the antibody, that exerts a special selective action upon the antigen, and either neutralizes its activity or prepares it for ultimate destruction. To this class of antibodies belong the agglutinins and precipitins, which agglutinate or precipitate their antigens preparatory, in a sense, to their final disintegration. For still more complex antigens nature has provided special ferment-like substances, always present in varying proportions in the blood, which, when united with the antigen, cause its disintegration and solution in a manner similar to the process of digestion as it takes place in the intestinal canal. These ferment substances are, however, powerless unless united with the antigens, and here we find that the antibody serves as the connecting link, binding antigen with ferment, which results in a form of digestion and final lysis or solution. The antibody is, therefore, simple in structure, and is composed of two binding or grasping arms—one for the antigen and one for a ferment. *This interbody, or amboceptor, is specific for the antigen, and will act only and specifically with this antigen.* It is important to remember that the ferment or complement is not an integral part of the antibody, but is free in the blood-stream; that the antibody is only a sensitizer or connecting link, but preserves its importance by being specific for its antigen; that the primary function of this antibody is to prepare (sensitize) the antigen, or unite antigen and complement, and that the latter then causes the lysis or solution of the antigen. The sensitizer or connecting link or antibody of this nature is known as an *antibody or receptor of the third order*.

Different cells produce their own and specific interbodies or amboceptors. Thus bacteria or vegetable cells, blood-corpuscles, and various other cells, such as ciliated epithelium, spermatozoa, renal epithelium, etc., when present in the form of an infection, or when injected into an animal, generate different and specific sensitizers or amboceptors, which bring about their solution by binding them with the ferment or complement. One ferment or complement may not serve for all; there may be various ferments, which act with the different amboceptors, but all have properties so nearly alike that many believe with Bordet that but a single complement exists.

Definition.—This special digestive and lytic process is known to occur with cells, and hence the antibodies capable of bringing about this action are called *cytolysins*, or *substances that cause lysis or solution of the various cells that may be their antigens*.

According to Ehrlich the three orders of antibodies each have their counterpart, both in structure and in effect, in the receptors serving for the normal nutrition of cells. For the simplest molecule of food that is in solution the cell is provided with a simple receptor for union with the molecule which is then directly assimilated. This receptor is similar to an anti-toxin, or an antibody of the first order, which destroys its toxin directly and without further ado. More complex food material must first undergo

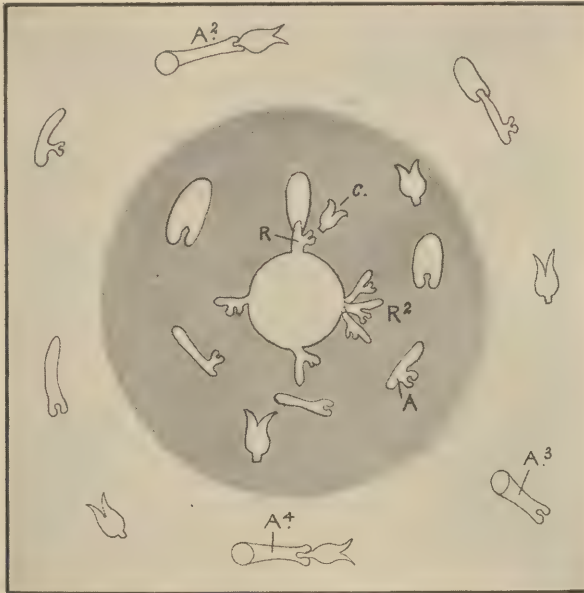


FIG. 115.—FORMATION OF CYTOLYSINS (HEMOLYSINS, BACTERIOLYSINS, CYTOTOXINS).

The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell.

R, Receptor of the molecule (*third order*); R², overproduction of receptors, which are being cast off; A, a cast-off receptor which now constitutes the antibody or amboceptor; C, molecule of complement free in the body cells and body fluids; A²A⁴, amboceptors in combination with molecules of a cell (antigen) and a complement; A³, an amboceptor in combination with a molecule of a cell. The cell (antigen) is now said to be sensitized. Lysis does not occur because a complement is not united.

some preparation by the cell before it can be assimilated, and accordingly we find receptors provided with a more complex structure which have their counterpart in the antibodies of the second order, or those possessing a special toxic portion that agglutinates or precipitates their antigen or prepares it for phagocytosis. It is possible that with physiologic substances this is all that the cell requires of its receptor, but so far as is known it would appear that for antibodies this action does not in itself injure the antigen, but is rather one step toward preparation for its further destruction. Organized and complex food substances must be digested before assimilation can occur, and here we find that the receptor acts as a link in binding the food molecule to a ferment, with resulting dissolution and assimilation of the products of solution. These are called receptors of the third order,

and have their counterpart in similar antibodies—the cytolytins—which act as links or interbodies between antigen and a complement, the latter being entirely free and separate, and independent of the receptor or antibody (interbody) itself (Fig. 115).

Varieties of Cytolytins.—The cytolytins produced by bacteria are known as *bacteriolysins*, *i. e.*, antibodies producing disintegration and lysis of bacteria. The cytolytins known as *hemolysins* cause lysis or hemolysis of the erythrocytes. Similar cytolytins may be formed for practically all cells, such as leukocytes, epithelium, liver, kidney, spleen, etc., and to these the general name *cytotoxin* has been given; thus we have leukotoxin, hepatotoxin, nephrotoxin, neurotoxin, etc., these terms being more nearly correct and expressive of the actual mechanism by which their action is produced.

Nomenclature.—In no other field of immunity have so many different names been applied to the same substances as have been applied to this order of antibodies. This confusion of terms, added to the various interpretations placed upon their significance, has rendered the subject incomprehensible to those not specially interested.

The ferment-like and thermolabile substances present in all serums and actively concerned in lytic processes have been given the name of *alexin* by Bordet; Metchnikoff called it *cytase*, and Ehrlich designated it as *complement* or *addiment* because in the conception of the side-chain theory it completes the reaction after being linked with the antigen. The term “alexin” was first applied by Buchner to the germicidal substance found in normal serum. We now know that Buchner was working with both bacteriolysins and complement, although Bordet was the first to discover the former, Buchner having been unconsciously most interested in the thermolabile complement.

To the antibody itself the term *substance sensibilisatrice* has been applied by Bordet, for he believes that this antibody sensitizes or prepares the cell for the action of the alexin or complement. The following names have been applied to the antibody by various observers: *fixative* or *fixateur*, by Metchnikoff; *preparator*, by Müller; and *amboceptor*, *interbody*, and *immune body* by Ehrlich. Of these the terms “sensitizer” and “amboceptor” are in most general use, signifying a two-armed body that unites antigen on the one hand, with a complement on the other.

When using the term “amboceptor,” care should be used to designate its specific character; thus, for example, a *hemolytic* amboceptor and a *bacteriolytic* amboceptor mean respectively a hemolysin and a bacteriolysin.

It is common practice to designate an amboceptor according to the cell for which it has a special affinity; thus *antisheep* amboceptor or hemolysin means an amboceptor for sheep cells, the prefix “anti” being affixed because it is specific for those cells.

AMBOCEPTORS OR SENSITIZERS

Although antitoxins have received considerable study from a therapeutic standpoint, probably no order of antibodies has been given more attention than the cytolytins have received, not only because of their vast therapeutic possibilities but also from their value as an aid to diagnosis. The hemolysins especially have been utilized in making the Wassermann test for syphilis and similar reactions, the very nature of the phenomenon offering a visible and fascinating method of study.

Since the general structure, formation, and action of the various sensitizers or amboceptors, such as the bacteriolysins, hemolysins, and other

cytolysins, are essentially similar, the general character of sensitizers may be here considered, a study of the special characteristics of each being reserved for subsequent chapters on the more important members of the group.

Historic.—The alexins or complements were first discovered through the researches of Nuttall and Buchner in 1889. The sensitizers were, of course, present in the various serums with which these observers were working, but it was not until 1895 that Bordet showed quite clearly that two substances were concerned in the phenomena of bacteriolysis and hemolysis. At this time he demonstrated that the alexin or complement may be removed from a serum by heating it to 55° to 56° C., and that it may be reactivated by the addition of fresh serum from another animal; that an old bacteriolytic serum cannot produce bacteriolysis unless it is reactivated by a fresh normal serum or is placed in the peritoneal cavity of a living animal, from which it may derive the thermolabile alexin. In other words, the sensitizers in these serums withstood the effects of heating and age, but were unable to produce lysis without the aid of an alexin furnished by a fresh normal serum.

Structure of Sensitizers or Amboceptors.—According to the theory of Ehrlich, an amboceptor is but a simple interbody furnished with two haptophore or grasping portions. One haptophore group attaches the antibody to its antigen whatever that may happen to be—bacterium, erythrocyte, epithelial cell, etc., while the other attaches a suitable complement (Fig. 115). The first is called the cytophil or antigenophil group, and the second, the complementophil group. The amboceptor is specific in the sense that it will unite only with its antigen or other very closely related body. For example, when a rabbit is injected with sheep corpuscles an amboceptor is formed that will unite only with sheep, and not with human, dog, ox, or other cells.

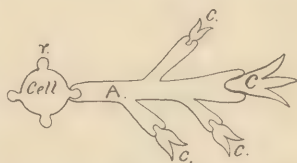


FIG. 116.—THEORETIC STRUCTURE OF A POLYCEPTOR.

A, Main portion of amboceptor in combination with a cell; C, dominant complement; c, lesser complements.

As will be shown further on, Ehrlich believes that many different complements may be present in a serum, whereas Bordet believes that one complement exists that will act with the sensitizer or amboceptor, whether this is bacteriolysin or hemolysin. This view is based mainly upon the observation that the complement in a serum may be absorbed out by furnishing an excess of either bacteriolytic or hemolytic amboceptors, the one variety of amboceptor removing all the complement for the other. Although the results of experimental work would seem to indicate that Ehrlich's belief in the plurality of complements is correct, and while this view is quite generally held, conclusive proof regarding this has not as yet been furnished. An amboceptor may have more than one complementophil group, and may bind a number of different complements simultaneously (*polyceptor*) (Fig. 116). Ehrlich and Morgenroth called attention to this possibility when they stated: "Finally, it is possible that an immune body besides one particular cytophil group, contains two, three, or more complementophil groups." Later Ehrlich and Marshall showed that in order to get a specific lytic effect it was not necessary for all complements to become active, but that only a few are necessary in any single instance to bring about effect. These complements are termed "dominant complements," the remainder being known as "non-dominant complements."

Amboceptoids.—Whether amboceptors can undergo degenerative changes and lose their cytophil or complementophil groups and become *amboceptoids*, just as toxoids and agglutinoids are formed, is still doubtful. Reasoning from analogy to the toxins and agglutinins, it is probable that amboceptoids may be produced by a loss of the complementophil group, the cytophil portion of all antibodies being more stable; such amboceptoids, by uniting with their antigens, may effectually block the action of an amboceptor, just as agglutinoids prevent agglutination.

General Properties of Sensitizers or Amboceptors.—Amboceptors are fairly resistant bodies, withstanding to a well-marked degree the effects of heat, acids, alkalies, exposure, and drying. A hemolytic serum, for instance, may be preserved in a sterile condition for many months and show but slight deterioration in its activity. Such a serum may be dried *in vacuo* or on suitable filter-paper, and preserve its activity for remarkable intervals of time with but slight and gradual deterioration. While a temperature of 55° C. will inactivate complement in from fifteen to thirty minutes, amboceptors can tolerate from 60° to 65° C. for an hour and show but slight depreciation in activity.

Formation of Sensitizers or Amboceptors.—While experimental data are at hand to show that amboceptors may be produced by local tissues, it is entirely probable that in wide-spread infection or as the result of artificial immunization there is general cellular activity with extensive antibody formation. The spleen and hematopoietic tissues in general and the mononuclear leukocytes are regarded by many as being particularly active in the formation of hemolysins and bacteriolysins (Pfeiffer and Marx, Deutsch, Wassermann).

As has been stated, Metchnikoff believes that antibodies of the class under consideration are the products of the leukocytes, thus tending to preserve the importance of the phagocytic theory. While there is little doubt that the various leukocytes, endothelial cells, and other phagocytic cells are sources of amboceptor production, there is no reason for accepting the belief that their formation is confined strictly to these cells.

Specificity of Amboceptors (Sensitizers).—It has been stated elsewhere in this volume that amboceptors are highly specific bodies. This specificity is not, however, absolute, for just as group agglutinins are produced by one bacterium for closely allied species, so in like manner experimental investigation by Ehrlich and Morgenroth, von Dungern, and others has shown that immunization of an animal with the erythrocytes of another animal would produce one chief hemolysin for these cells and a secondary hemolysin for the cells of another animal. For example, on immunizing a rabbit with ox blood a hemolytic serum was obtained that was hemolytic not only for goat blood but also for ox blood. These secondary amboceptors are known as *group* or *partial immune bodies*. Their production may readily be understood when it is remembered that the body cells are conceived as being provided with various side arms for many different blood-cells, bacteria, etc. Now, if the erythrocytes of the goat possess receptors not only for the particular goat-blood side arms of the body cells but also for the ox-blood side arms, both sets of side arms will be attacked and consequently two amboceptors are formed—one, the main one, for goat corpuscles, and a secondary one for ox corpuscles. Ehrlich and Morgenroth, therefore, claim that the immune body of a hemolytic serum is composed of the sum of the partial immune bodies, which correspond to the individual receptors used to confer the immunity. Since the cells of various animals of the same and of different species vary in the number and variety

of side arms or receptors, which are not present in another, the different combining group possessed by a blood-cell or a bacterium will not, therefore, find fitting receptors in every animal, and thus there may be a different variety of partial immune bodies in two animals. This would lead to the possibility of the occurrence of antibodies for the same blood-cell or bacterium, differing from one another in the partial immune bodies of which they are composed, depending on the variety of the animals used in preparing the serum.

This view is directly opposed to that of Metchnikoff and Besredka, who believe that a certain immune body is always the same no matter what species of animal was used in preparing the serum. As will be pointed out further on, in addition to theoretic interest, the subject possesses great practical importance, for as is well known, most curative serums are best prepared with many different strains of a particular micro-organism because of certain differences in their antigenic properties, and if, in addition, the value of a bacteriolytic serum depends upon the sum total of the immune bodies it may be advisable to secure as many of these as possible by preparing the serum from various animals of the same and of different species.

It will be understood, therefore, that the specific action of antibodies of this order is not limited to the cells used in the immunizing process, but extends to other cells that have receptors in common with these, a condition that is analogous to group agglutinins and precipitins for closely allied cells and bacteria or dissolved albumins.

Natural or Native Amboceptors.—Just as small and varying amounts of native agglutinins and antitoxins may be found in normal serums so, in like manner, various native bacteriolytic and hemolytic amboceptors may be found. According to the side-chain theory, these various amboceptors are normally attached to body cells, hence it is probable that a few are being continually swept off into the blood-stream. In some instances the amount of a natural amboceptor may be quite high; thus, for example, many human serums contain relatively large amounts of antishoop hemolytic amboceptor. These natural amboceptors will be considered more fully in the chapters on Hemolysins and Bacteriolysins.

The difference between a normal and an immune serum lies in the fact that the normal serum contains a number of amboceptors in small amounts, whereas the immune serum contains a greatly increased amount of at least one amboceptor for a particular cell. As has been shown by numerous investigators, this difference is not due to the complements, as these are not increased during the process of immunization. Since the presence of an amboceptor cannot be demonstrated unless complement is present, in testing a serum for an amboceptor we must furnish sufficient complement to bring out the maximum activity of the amboceptor. If the serum of a rabbit before and after immunization is titrated with sheep erythrocytes, it may be found that the immune serum contains from a hundred to many thousand times the normal quantity of antishoop amboceptor.

These facts bear a further practical relation to the treatment of infectious diseases with bacteriolytic serums. Ordinarily, when we inject an immune serum we furnish but one bactericidal substance, namely, the bacteriolytic amboceptor, and no complement at all. If the patient's complement is decreased or at least insufficient to activate the amboceptor furnished, lysis will not occur, and accordingly an increased therapeutic effect may be secured by the injection simultaneously of an immune serum and a fresh normal serum. This procedure presents certain difficulties

and the subject is considered more fully in the chapter on Passive Immunization.

Antiamboceptors.—Just as antiagglutinins and antiprecipitins may be formed, so antiamboceptors may be produced experimentally by immunizing an animal with an amboceptor-laden serum. An antiamboceptor is specific for the amboceptor that caused its production, and when these are mixed the activity of the amboceptor is impeded by the antiamboceptor, which unites with its cytophilic group. It is possible that old erythrocytes are destroyed by an autohemolysin present in the blood-stream under normal conditions, and that a physiologic equilibrium is maintained through the production of an antiamboceptor.

COMPLEMENT OR ALEXIN

Historic.—As early as 1876 Landois described the hemolytic action of fresh blood-serum upon the blood-corpuscles of animals of certain species. Traube and others observed that animals could withstand the injections of relatively large amounts of septic material, but it was not until 1886-90 that Fodor,¹ Nuttall,² Buchner,³ and others fully established the bactericidal properties of fresh blood-serum. Buchner demonstrated the fact that the active principle causing bacteriolysis or hemolysis is very labile, and can be inactivated by a temperature of 55° C., by dialysis, or by dilution with distilled water. He designated the active principle "alexin," which means "protective substance."

Subsequently, in 1899, Bordet found that the alexin of Buchner was composed of two distinct substances—one a sensitizing substance, which is thermostable, and a second, the thermolabile substance. Somewhat later (1899) Ehrlich and Morgenroth confirmed these observations, but applied the name "amboceptor" to the sensitizing substance and "complement" to the alexin. These terms are most widely employed at the present time. Bordet adheres to the term "alexin," meaning thereby the thermolabile principle, and does not use it in the original sense of Buchner, which included both the sensitizing substance and the alexin. Metchnikoff's cytases are practically the same as Ehrlich's complement and Bordet's alexin.

Definition.—*Complement or alexin [Lat., complementum, that which completes] is the substance, present alike in normal and in immune serum, which is destroyed by heating to 55° C., and which acts with an amboceptor or sensitizer to produce lysis.*

As mentioned in the discussion on amboceptors, the complement is the active lytic substance concerned in the phenomenon of cytolysis, but is powerless until united with the cell, corpuscle, or bacterium by means of the interbody or amboceptor, that is, with a sensitized antigen.

Structure and General Properties of Complement.—Complement is ordinarily not attached to the body cells and is free in the blood-serum. According to Ehrlich, complement is simple in structure, and is composed of a haptophore portion for union with the complementophil haptophore of an amboceptor, and a second toxic or lytic portion, called the cytolytic group. In other words, the theoretic structure is similar to that of a toxin, although the function and action of the two are quite different.

As will be discussed later, various investigators have claimed that complement may be split or fractionated into two portions, the midpiece being

¹ Deutsch. med. Wchn., 1886, 617.

² Ztschr. f. Hyg., 1888, 4, 353.

³ Arch. f. Hyg., 1890, 10, 84.

identified with the haptophore portion or that which unites with a sensitized antigen, and the end piece with the active or cytolytic portion.

Effect of Heat Upon Complement; Inactivation of Complement; Complementoids.—The extreme sensitiveness of complement to heat is one of its prominent characteristics; as a general rule, heating a fresh serum in a water-bath at 55° C. for ten or fifteen minutes results in the complete inactivation of hemolytic complement, although heating for thirty minutes is commonly employed for this purpose.

Complement deteriorates rapidly after bleeding unless the serum is frozen. At ordinary room temperature deterioration is rapid within twenty-four hours, and generally complete within a few days.

When a serum contains complement it is said to be *active* and this must, under ordinary circumstances, be a fresh serum. On heating or exposure, the serum becomes *inactivated*; an inactivated serum may be reactivated by the addition of fresh serum.

Inactivation of hemolytic complement is commonly believed to be due to inactivation of the cytolytic or active portion of the molecule which has been designated by Ehrlich as *complementoid*.

Inactivation by heating, shaking, or standing is apparently not an irreversible process as commonly believed. Gramenitski¹ has observed a gradual return to an active condition after moderate heating, the following protocol being one published by him showing the effect of heating at 56° C. upon 1 : 10 complement and tested against sensitized beef corpuscles:

TIME AFTER HEATING AT WHICH TEST WAS MADE.	HEMOGLOBIN GONE INTO SOLUTION AFTER			
	Per cent., ten minutes.	Per cent., twenty minutes.	Per cent., thirty minutes.	Per cent., forty minutes.
Seven minutes.....	..	20	40	70
One and a half hours.....	..	30	60	80
Twenty-four hours.....	20	70	80	100
Forty-eight hours.....	10	40	70	

The largest amount of reactivated complement seemed to be present after twenty-four hours, followed by gradual deterioration. Gramenitski has explained this phenomenon on the basis of Traube's² work, showing that when a serum is heated at 56° C. there is a reduction of surface tension due to alteration of colloidal condition, that is, an aggregation of particles which, if not carried too far, may be reversible and followed by dispersion and reactivation as the serum is kept. I have been able to confirm the essential particulars of Gramenitski's work on this interesting phenomenon.

Since complementoids have their haptophore groups intact they will unite with amboceptors and to some extent prevent lysis by blocking the active complement just as toxoids unite with antitoxin and agglutinoids with their antigens.

Effect of Acids, Salts, and Filtration Upon Complement.—Hemolytic complement is extremely susceptible to the effects of acids and alkalis; for this reason all glassware employed in tests employing complement must be scrupulously clean. Nolf,³ von Lingsheim,⁴ Hektoen and Rue-

¹ Biochem. Ztschr., 1912, 38, 504.

² Ztschr. f. Immunitätsf., 1911, 9, 246.

³ Ann. d. l'Inst. Pasteur, 1900, 14, 297.

⁴ Ztschr. f. Hyg., 1901, 37.

diger,¹ Manwaring,² von Dungern and Herschfeld³ have shown that many inorganic salts in small amounts destroy or inactivate complement, and Cumming,⁴ Brown and Kolmer,⁵ Sherwood,⁶ and others, have shown the extreme destructiveness of acids and alkalies.

The manner in which this inactivation or destruction is brought about is obscure. Strangely enough the inactivation by salt may be temporary, that is, full activity is restored when the solution is reduced to isotonicity, as shown by Muir and Browning.⁷ These investigators have also shown that complement inactivated by the addition of 5 per cent. sodium chlorid passes through Berkefeld filters, whereas plain complement serum is held back, probably by a process of absorption. Kyotoku, in my laboratory, has also shown that hemolytic complement of guinea-pig-serum is not filterable if fresh, clean filters are employed, until a large amount of serum has been passed, confirming the work of Muir and Browning. Early investigations by Ehrlich and Morgenroth,⁸ and Vedder⁹ were to the effect that some complements were filterable and others not. In all probability a great deal depends upon the kind of filter employed and the influence of sodium chlorid upon inactivation and filterability of complement is probably one of colloidal dispersion.

Effect of Shaking Upon Complement.—Jacoby and Schütze¹⁰ have shown that guinea-pig-serum complement is readily destroyed or inactivated by shaking and have pointed out the possible influence of this factor upon complement-fixation tests. Ritz,¹¹ Kashisabara,¹² Schmidt,¹³ and others, have confirmed these observations. Noguchi and Bronfenbrenner,¹⁴ however, found that shaking for one hour at 37° C. had almost no influence and conclude that the several shakings necessary for complement-fixation tests do not appreciably influence the reactions.

Anticomplements.—Ehrlich and Morgenroth have claimed that these may be obtained by immunizing suitable animals with serums that contain complement, or complementoid. When an inactivated anticomplement serum is mixed with the homologous complement, the haptophores of the latter are bound by means of the haptophores of the anticomplements. A proof of this union lies in the fact that a complement serum that has been treated with its specific anticomplement is no longer able to activate an appropriate amboceptor.

According to Gay, the production of anticomplements is only apparent; he explains the loss of complement activity when a fresh serum and its antiserum are mixed as due to the absorption of complement in the precipitate which forms, although the latter may be invisible.

Anticomplements may be of practical importance owing to the formation of *auto-anticomplements*. The complements must exercise an important function, not only in the destruction of bacteria, but also in the digestion and solution of all kinds of foreign albuminous bodies that enter the organism. As was shown by Wassermann, anticomplements may so bind up their complements as to render their host much less resistant to certain infectious diseases. The spontaneous development of auto-anticomplement in an animal has never been demonstrated, as there are no receptors in an organism of the complements of the same organism. The injection of the

¹ Jour. Infect. Dis., 1904, 1, 379.

² Jour. Infect. Dis., 1904, 1, 112.

³ Ztschr. f. Immunitätsf., 1911, 10, 131.

⁴ Jour. Infect. Dis., 1916, 18, 151.

⁵ Amer. Jour. Syph., 1919, 3, 8.

⁶ Jour. Infect. Dis., 1917, 20, 185.

⁷ Jour. Path. and Bact., 1909, 13, 76.

⁸ Berl. klin. Wchn., 1900, Nr. 31, 682.

⁹ Jour. Med. Research, 1903, 9, 475.

¹⁰ Ztschr. f. Immunitätsf., 1910, 4, 730.

¹¹ Ztschr. f. Immunitätsf., 1912, 15, 145.

¹² Ztschr. f. Immunitätsf., 1913, 17, 21.

¹³ Ztschr. f. Immunitätsf., 1913, 19, 373.

¹⁴ Jour. Exper. Med., 1911, 13, 229.

serum of another animal containing complements that are almost identical may, however, lead to the formation of an auto-anticomplement in the serum of the immunized animal.

Source of Complement.—Despite a great amount of investigation upon this subject, owing to its interest and importance, the source or sources of complement production remains in doubt.

Hankin,¹ and shortly afterward Kanthack and Hardy,² advanced the hypothesis that complement was a secretory product of the eosinophil leukocytes, but this theory could not be supported by solid arguments or experimental data. A similar theory was proposed by Buchner,³ who held that it is not the eosinophils only that secrete the alexins, but the leukocytes in general. Later Hahn,⁴ Schattenfroh,⁵ Laschtschenko,⁶ and Trommsdorff⁷ sought to confirm this theory by exact experiments and the sum total of their work justifies one in believing that the living leukocytes are at least one source of complement production.

Metchnikoff,⁸ however, denied that living leukocytes secrete this substance and maintained that alexin or complement was not present in the plasma and was produced by leukocytes and other body cells only upon leukocytic injury and disintegration or phagolysis. He was led to adopt this view by reason of the experiments of Gengou,⁹ showing that alexin or complement was not present in plasma. The work of Hahn,¹⁰ and more recently and especially that of Hewlett,¹¹ Lambotte,¹² Addis,¹³ Dick,¹⁴ and Watanabe¹⁵ has shown, however, quite conclusively that complement is present in plasma where its presence is probably due to continual disintegration of leukocytes and liberation of complement during life. It is apparently increased to a slight extent, as serum is left in contact with the blood-clot as shown by Walker,¹⁶ Gurd,¹⁷ Kolmer,¹⁸ and others, indicating that disintegration of leukocytes may augment the complement supply. Gengou,¹⁹ however, has recently shown that the endolysins or bacteriolytic substances obtained by the disintegration of leukocytes, do not have the properties of alexin or complement. It is highly probable that leukocytes contain or elaborate both as separate entities.

The failure to obtain definite proof of the origin of complement in the leukocytes has led to search for the source in various organs and especially the liver. Morgenroth and Ehrlich²⁰ observed a diminished amount of complement in dogs subjected to phosphorous poisoning and Nolf, by means of extirpation experiments with rabbits, found an extreme reduction. These results were confirmed by Müller,²¹ but Liefmann,²² who repeated Müller's experiments, obtained negative results and especially with frogs. Dick,²³ however, as a result of experiments of this nature concluded that comple-

¹ Centralbl. f. Bakteriöl., 1892, 12, 777, 809; *ibid.*, 1893, 14, 852.

² Proc. Roy. Soc. Med. London, 1892, lii, 267.

³ Münch. med. Wchn., 1894, 717 and 1897.

⁴ Arch. f. Hyg., 1895, 25, 105; *ibid.*, 1897, 28, 312.

⁵ Arch. f. Hyg., 1897, 31, 1; *ibid.*, 1899, 35, 135.

⁶ Arch. f. Hyg., 1900, 37, 290.

⁷ Arch. f. Hyg., 1901, 40, 382.

⁸ Immunity in Infective Diseases, 1905, 190 et seq.

⁹ Ann. d. l'Inst. Pasteur, 1901, 15, 232.

¹⁰ Arch. f. Hyg., 1895, 25, 105.

¹¹ Arch. f. exper. Path. u. Pharmakol. 1903, 49, 307.

¹² Centralbl. f. Bakteriöl., 1903, 34, 453.

¹³ Jour. Infect. Dis., 1912, 10, 200.

¹⁴ Jour. Infect. Dis., 1913, 12, 111.

¹⁵ Jour. Immunology, 1919, 4, 77.

¹⁶ Jour. Hyg., 1903, 3, 52.

¹⁷ Jour. Infect. Dis., 1912, 11, 225.

¹⁸ Amer. Jour. Syph., 1919, 3, 407.

¹⁹ Ann. l'Inst. Pasteur, 1921, 35, 497.

²⁰ Berl. klin. Wchn., 1900, 37, 683.

²¹ Centralbl. f. Bakteriöl., 1911, 57, 577.

²² Weichhart's Jahresbericht, 1912, 8, 155.

²³ Jour. Infect. Dis., 1913, 12, 111.

ment is formed in the liver or is dependent upon this organ for its presence in the blood. Fassin¹ found that extirpation of the thyroid gland was followed by a decrease of complement, while the subcutaneous injection of the extract to dogs and rabbits was followed by a rapid increase; Dick also observed a decrease after thyroidectomy. These results, however, are not conclusive, as it may well be that the thyroid is concerned in stimulating the production of complement by other tissues without itself being an important source of origin.

Multiplicity of Complements.—Ordinarily a fresh serum, such as that of the guinea-pig, will furnish complement for either bacteriolytic or hemolytic amboceptors, and the question arises as to whether one complement unites equally well with all amboceptors, or whether several complements are present in one serum that act more or less specifically with different amboceptors.

The question is whether one and the same serum may contain more than one alexin or complement and not whether the alexins or complements in the sera of different animals are functionally identical, as it is well known that the complements of different animals of the same and different species vary in their power to activate bactericidal and hemolytic systems.

Bordet² believes that only one complement is present, and bases this opinion mainly on the fact that a complement that can be shown to activate either a hemolytic or a bacteriolytic amboceptor may be absorbed out of a serum by furnishing an excess of either amboceptor.

Metchnikoff³ maintains that there are two cytases or complements, one being derived from macrophages and mainly hemolytic, and the second derived from microphages and chiefly bacteriolytic.

Ehrlich and Morgenroth,⁴ Sachs,⁵ Wassermann,⁶ Wechsberg,⁷ and the German school in general believe that many different complements are present in amounts varying with the different serums. These observers have sought to prove this experimentally, and while the evidence is not absolutely convincing, because of the difficulty of working with substances that are so labile, yet the doctrine of the multiplicity of complements is quite generally accepted on the basis of such data, as follows:

(a) By digesting 20 c.c. of fresh goat-serum that was found to activate different hemolytic amboceptors with 3 c.c. of a 10 per cent. solution of papain in the incubator for from thirty to forty-five minutes, it was found that the complement for one amboceptor was destroyed, whereas those remaining were left intact or but slightly impaired.

(b) By treating 10 c.c. of this goat-serum with 1 c.c. of a 7 per cent. solution of soda for an hour it was found that some complements were destroyed and others were weakened.

(c) By sensitizing different blood-cells with homologous amboceptors and adding these to a fresh serum for short and varying periods of time, some complements could be absorbed, whereas others would be left behind with undiminished or but slightly decreased activity. Prolonged exposure would remove all complements.

(d) As was previously stated, anticomplements may be produced by immunizing an animal with the complement of an animal of a different

¹ Compt. rend. Soc. de biol., 1907, 62.

² Ann. d. l'Inst. Pasteur, 1900, 14, 257.

³ Immunity in Infect. Dis., 1905, 185 et seq.

⁴ Berl. klin. Wchn., 1900, 453, 677.

⁵ Berl. klin. Wchn., 1902, No. 21.

⁶ Ztschr. f. Hyg., 1901, 37.

⁷ Ztschr. f. Hyg., 1902, 39.

species. The anticomplements appear to be specific for the complements responsible for their production, and by means of these anticomplements different complements may be demonstrated in one serum. Since the formation of anticomplements would depend upon whether or not the body cells of the immunized animal possess suitable receptors for the various complements, in a series of animals it may be found that one does not produce anticomplements for all the complements injected, a finding that would tend to support the theory of the multiplicity of complements. In addition, Marshall and Morgenroth actually found in ascitic fluid an anti-complement for at least one of two complements present in guinea-pig serum.

(e) By passing normal goat serum through Pukall filters, a complement for sensitized guinea-pig cells passed, whereas a complement for sensitized rabbit corpuscles was withheld.

By these and other experiments, including careful heating, Ehrlich and his school sought to prove that many different complements are to be found in normal serum. Neisser¹ was among the first to adopt the view on the basis of his experiments that treatment of rabbit-serum with anthrax bacilli removed bacteriolytic complement, but not a complement acting upon sensitized goat and sheep corpuscles. Wilde,² however, refuted these results and showed that if a sufficient excess of bacilli were employed all of the complement could be removed; Bordet³ observed exactly similar results when serum was treated with an excess of sensitized corpuscles, that is, all of the alexin or complement was absorbed.

These experiments go to show that complements differ in this respect at least: that not all have identical haptophores. Whatever differences between complements exist must be slight; probably the cytophilic group of all are alike. At present the subject has more theoretic than practical importance. In the various diagnostic reactions guinea-pig-serum ordinarily furnishes the complement for hemolysin, bacteriolysin, or other cytolytins, and in the therapeutic administration of bacteriolytic serums we are compelled in any case to depend for activation of the amboceptor upon the natural complement in the patient's serum.

Variation in Amount of Complement in Serum.—The complement content of the sera of different animals varies within wide limits, and particularly that complement acting with sensitized blood-corpuscles, as shown by Noguchi and Bronfenbrenner,⁴ Kolmer, Matsunami, and Trist,⁵ and others. For example, the average human complement is five to seven times less hemolytic for sensitized sheep corpuscles than the average guinea-pig complement, and the sera of the white rat, rabbit, swine, sheep, and ox show similar wide variations.

Even among animals of the same species slight variations in the complement content of the sera have been found, and, indeed, in the serum of the same person or lower animal at different times during the day.

In addition to these quantitative variations in the complement content of normal sera, widely different qualitative variations are known to occur and especially in relation to the complement-fixation test. For these reasons it is customary to use a mixture of sera for complement in conducting these tests in order to reduce the chances of error.

Apparently wide variations in the complement content of human sera occur in disease. These changes have been largely studied by Gunn⁶ and

¹ Deutsch. med. Wchn., 1900, 790.

² Berl. klin. Wchn., 1901, No. 34.

³ Ann. d. l'Inst. Pasteur, 1901, 15, 303.

⁴ Jour. Exper. Med., 1911, 13, 78.

⁵ Amer. Jour. Syph., 1919, 3, 407.

⁶ Jour. Path. and Bacteriol., 1914-15, 19, 155.

others by titrating the serum with sensitized blood-corpuscles on the basis of Bordet's work, that with an excess of sensitizer the total complements (bacteriolytic as well as hemolytic) may be measured. In typhoid fever Gunn observed that the amount of complement bore some relation to the severity of the disease, being least or weakest in patients who are very ill. Similar studies were made in erysipelas, diphtheria, and scarlet fever.

The Nature of Complements.—The true nature of the complements is unknown. In many respects they bear a resemblance to ferments, and certainly the part they play in the processes of cytolysis suggests a ferment-like activity. Buchner,¹ Bordet,² Ehrlich and Morgenroth,³ and other of the pioneer investigators have subscribed to this view; Metchnikoff believed that the evidence indicated that alexin is one of the numerous intra-leukocytic soluble ferments which passes into the fluids as the result of rupture or of damage to the phagocytes. Liefmann,⁴ who has studied the nature of complement very extensively, has concluded that most evidence supports the ferment hypothesis on the nature of complement. The chief objection to this view has been based upon the fact that complement, unlike the enzymes, is used up during cytolysis and that a definite quantitative relationship exists between the complement and sensitized cells upon which it acts. Recent experiments by Kiss,⁵ however, have shown that this quantitative relationship is not as strict as formerly supposed, and that the action of complement depends very largely upon its concentration, which strengthens the hypothesis of the ferment nature of complements. They differ from true proteolytic ferments such as trypsin in not digesting the stroma of corpuscles, although recent work by Dick⁶ would seem to indicate that proteolysis actually occurs, a process that increases the permeability of the cell and permits the escape of hemoglobin.

On the other hand, it is possible that the nature and action of complements may be placed upon a chemical basis. Following the discovery of the hemolytic power of cobra venom by Flexner and Noguchi,⁷ a power they ascribed to the presence of an amboceptor in the venom acting with serum complement, Kyes⁸ found that the amboceptor may be activated not only by a complement in the blood-serum but also by some constituent of the red blood-corpuscles themselves. This last observer speaks of the latter as *endocomplement*, *i. e.*, endocellular complement.

In attempting to discover the nature of this endocomplement various substances existing normally in the erythrocytes, such as cholesterin and lecithin, were obtained in a pure state and their activating powers for cobra amboceptors tested. These investigations showed that lecithin has an activating power, whereas cholesterin is antihemolytic. Although all erythrocytes contain lecithin, yet all are not equally susceptible to the action of venom amboceptors, which is probably due to the fact that the lecithin in the cells of some animals is bound to other cell constituents in a loose way and is thus available as complement. In syphilitic infection the lecithin content of the erythrocytes is actually diminished or in some manner rendered less available, so that the inhibition or absence of venom hemolysis is characteristic of this infection.

¹ Münch. med. Wchn., 1900, 1193.

² Ann. d. l'Inst. Pasteur, 1898, 12, 688; *ibid.*, 1899, 13, 273.

³ Berl. klin. Wchn., 1899, 6, 481.

⁴ Ztschr. f. Immunitätsf., 1913, 16, 503.

⁵ Ztschr. f. Immunitätsf., 1909, 3, 558.

⁶ Jour. Infect. Dis., 1913, 12, 111.

⁷ Jour. Exper. Med., 1902, 6, 277.

⁸ Berl. klin. Wchn., 1902, Nos. 38, 39; *ibid.*, 1903, Nos. 2-4.

Kyes was able to obtain the union of cobra amboceptor and lecithin, forming what is known as *cobra lecithid*. Although lecithin is an unstable substance and is difficult to obtain free from fatty acids and soap, there is little doubt that Kyes' lecithid is a phosphatid compound and is actively hemolytic after all traces of fatty acids have been removed.

The next important observations were made by Noguchi,¹ who found that soap isolated from blood and various tissues possessed active hemolytic properties. The salts of the fatty acids, and particularly of oleic acid, were found to possess similar hemolytic properties. Pure soluble oleates mixed with serum were found to produce compounds possessing many of the characteristics of true complements: (1) They are inactivated by heating to 56° C. for half an hour; (2) they are inactive at 0° C.; (3) the addition of acids, alkalies, and yeast renders them inactive. Von Liebermann,² as the result of his own experiments, came to practically the same conclusions, and advanced the hypothesis that the complements of the blood are to be sought for in the soaps of the serum; that these soaps are united with serum albumin, and are inactive until liberated by the amboceptors, when they become actively hemolytic. Objections were soon raised, however, by Hecker,³ Friedeman and Sachs,⁴ and Knaffi-Lenz,⁵ whose experiments tended to show that the hemolytic action exerted by fatty acids or soap is quite incomparable to true complement action and are thermostable. Von Dungern and Coca⁶ have also shown that cobra venom contains a lipoid-splitting ferment which acts upon the lecithin, liberating hemolytic substances of a non-specific nature. Sachs and Altmann,⁷ Liefmann, Cohen, and Orloff,⁸ and others are likewise opposed to the lipoidal nature of the complements.

Of further interest in this connection it was shown that oleic acid may act as an amboceptor, and when added to an inactive soap-albumin combination it would render this actively hemolytic. Von Liebermann and Fenyvessy⁹ have shown that a mixture of soap, serum, and oleic acid possesses a striking resemblance to complements and amboceptors, and that the amboceptor-complement action is much more than a mere linkage of complement to antigen by means of an amboceptor. These observers suggest that an amboceptor may have an affinity for certain constituents of the cell or bacterial body, and, on the other hand, act upon the complement and separate one of its constituents, which then breaks up the cell. These artificial hemolysins, however, completely dissolve the stroma of the corpuscles, whereas the immune hemolysins appear to dissolve out the hemoglobin, leaving the stroma undissolved. As has been mentioned elsewhere, recent work would tend to show that the stroma is also dissolved, at least in part, in specific hemolysis, so that the difference in action between the two is not quite so apparent.

While the simplicity of the substances concerned in these observations does not harmonize with the great variety and complexity of the immune bodies, nevertheless, as Adami has pointed out, the points of resemblance between artificial and natural complements and amboceptor are so strik-

¹ Proc. Soc. Exper. Biol. and Med., 1907, 4, 107; Biochem. Zeitschr., 1907, 6.

² Biochem. Zeitschr., 1907, 4; Ztschr. f. Immunitätsf., 1912, 13, 695.

³ Arb. a. d. k. Inst. f. Exper. Therap., 1907, 3.

⁴ Biochem. Ztschr., 1908, 12.

⁵ Biochem. Ztschr., 1909, 20.

⁶ Berl. klin. Wchn., 1907, 46.

⁷ Berl. klin. Wchn., 1908, 10.

⁸ Ztschr. f. Immunitätsf., 1912, 13, 150.

⁹ Biochem. Zeitschr., 1907, 5; Ztschr. f. Immunitätsf., 1911, 10, 479.

ing that material advances in our knowledge of their nature and action may be gained by further researches into the chemistry of immunity.

Complement Splitting.—The inhibiting effect of hypertonic (5 per cent.) solution of sodium chlorid upon the activity of complement and its filterability has been discussed in preceding sections; mention should also be made of the early observations of Buchner and Orthenberger¹ to the effect that bacteriolysis is inhibited in the complete absence of salts. Some years later Ferrata² showed that this inhibition of cytolysis was due to a failure of function on the part of complement and not to any failure of union of sensitizers or amboceptors with the antigens. This inactivation of complement in distilled water was ascribed to disturbances of colloidal equilibrium or to the destruction of complement by a ferment which was believed by Sachs and Teruuchi³ to be active in a salt-free medium.

Ferrata removed the salts by dialyzing for twenty-four hours against distilled water. This resulted in "splitting" the serum into a precipitate of globulins and the water-soluble albumins. By dissolving the former in isotonic saline solution and rendering the latter isotonic by the addition of salt, Ferrata found that neither portion was lytic, whereas a mixture of the two was actively lytic for sensitized corpuscles.

These observations upon the splitting of complement were soon confirmed and extended by Brand,⁴ who suggested the terms "midpiece" for the globulin fraction, because it unites with the sensitized antigen, and "end piece" for the albumin portion, because it cannot combine directly with the amboceptor and antigen, but is bound only after the globulin fraction has been previously attached.

Brand found both portions thermolabile while free, but Michaelis and Skwirsky⁵ showed that the globulin or "midpiece" when united with sensitized antigen, was no longer affected by heating at 56° C. Hecker⁶ found that while the "midpiece" or solution of globulins in water became inactive after three or four hours, yet it was capable of uniting with sensitized antigen, and that when "end piece" or albumin fraction was subsequently added hemolysis occurred. This phenomenon has not been adequately explained. Hecker and, later, Sachs have suggested that "midpiece" or globulin fraction when inactivated by standing loses its avidity for the sensitized antigen, but acquires an increased combining power for the "end piece." This theory, however, lacks proof, and in all probability the true mechanism is concerned with colloidal phenomena.

Of great interest in connection with this subject of complement splitting is the question whether complement or alexin in normal serum exists as a combination of mid- and end pieces or as separate fractions. A definite answer cannot be given. Apparently the two portions may be absorbed separately from serum by sensitized blood-cells; Michaelis and Skwirsky, for example, have found that while acid reaction inhibits hemolysis, yet "midpiece" is bound, but the "end piece" or albumin fraction is unbound. They have recommended the use of strongly sensitized corpuscles on an acid medium as a method for securing the albumin fraction or "end piece" in pure form by removing the "midpiece" after union with sensitized corpuscles by centrifuging. This would indicate the separate existence of these complement partitions in serum, but experiments are rendered difficult by

¹ Arch. f. Hyg., 1890, 10, 149.

² Berl. klin. Wchn., 1907, xlv, 366.

³ Berl. klin. Wchn., 1907, xlv, 467, 520, 602.

⁴ Berl. klin. Wchn., 1907, xlv, 1075.

⁵ Ztschr. f. Immunitätsf., 1909-10, 4, 357, 629.

⁶ Arb. a. d. k. Inst. f. Exper. Therap., 1907, No. 3, 39.

reason of the fact that traces of "end piece" or the albumin fraction carried down with the "midpiece" or globular fraction will activate the latter, which would indicate that in the serum both portions are probably present in combination or as a complex. To secure the fractions in purer form other means of fractionating have been used in preference to the dialyzation method. Thus Sachs and Altman¹ precipitate with $n/300$ to $n/250$ hydrochloric acid. Fränkel² dilutes the serum ten times with distilled water and introduces carbon dioxid. Salting out methods are unsatisfactory because of the prolonged dialysis necessary for the removal of the salts. With these various methods it has been shown by Liefmann and Cohn,³ Marks,⁴ and others that quantitative factors are of great importance in a study of the action of these portions separately and together; all that can be regarded as demonstrated is that under certain circumstances complement may be separated into at least two parts; that one part largely identified with the globulin fraction, acts with the sensitized antigen, rendering it amenable to the lytic activity of the albumin fraction.

This is not, however, accepted by all investigators.

Bronfenbrenner and Noguchi⁵ have cast considerable doubt upon these views, and have shown that what is known as complement splitting is really nothing more than an inactivation of the active principle of complement, since both globulin and albumin fractions contain a part of the complement, a fact that can be demonstrated by the removal of the inhibiting action of the acid or alkali used in the process.

The Bordet-Gengou Phenomenon of Complement Fixation.—In the endeavor to demonstrate the unity of complement Bordet and Gengou⁶ devised an experiment that has proved of great practical value in the serum diagnosis of syphilis and other infectious diseases. By mixing bacteria and their amboceptors with a little fresh serum containing complement and letting the mixture stand aside for an hour or so it was found that, upon the addition of corpuscles and their amboceptor, hemolysis did not occur, although the serum that had been used as complement was capable, in its original condition, of producing hemolysis of these corpuscles. Bordet advanced this experiment to show that the complement concerned in bacteriolysis is the same as that at work in hemolysis, and consequently concluded that there is but one single complement.

This experiment of Bordet is usually spoken of as the "Bordet-Gengou phenomenon," and is now used extensively in determining whether or not a given serum contains certain amboceptors. The serum to be tested is first inactivated, treated with the antigen composed of an emulsion of the bacterium whose amboceptor it is desired to discover, and then mixed with a small quantity of a fresh normal complement serum. The mixture is placed in the incubator for an hour, during which time the bacterial antigen unites with its amboceptor, and the complement, *i. e.*, fixes the complement, so that when red blood-cells previously sensitized with heated hemolytic serum are added, hemolysis does not occur because the complement in the fresh serum, which was suitable for lysis of the sensitized corpuscles, has been "fixed" by the bacteria by reason of the presence of specific amboceptors in the serum tested (Fig. 117). If these amboceptors were

¹ Berl. klin. Wchn., 1908, xlv, 699.

² Ztschr. f. Immunitätsf., 1911, 8, 781; *ibid.*, 1911, 10, 388.

³ Ztschr. f. Immunitätsf., 1910, 6, 562; *ibid.*, 1910, 7, 699.

⁴ Ztschr. f. Immunitätsf., 1911, 8, 508; *ibid.*, 1911, 11, 18. Jour. Exper. Med., 1911, 13, 590.

⁵ Jour. Exper. Med., 1912, 15, 598, 625.

⁶ Ann. d. l'Inst. Pasteur, 1901, 15, 289.

not present, then the complement would remain unfixed and be free to hemolyze the sensitized corpuscles, a negative reaction being indicated, therefore, by hemolysis, whereas the absence or inhibition of hemolysis indicates a positive reaction. A more complete description of this interesting phenomenon is given in Chapter XXIII.

Wassermann, Neisser, and Bruch have applied this test to the serum diagnosis of syphilis, the technic of the test being considered in a subsequent chapter.

The Neisser-Wechsberg Phenomenon of Complement Deviation.—This is frequently confused with the phenomenon of complement fixation

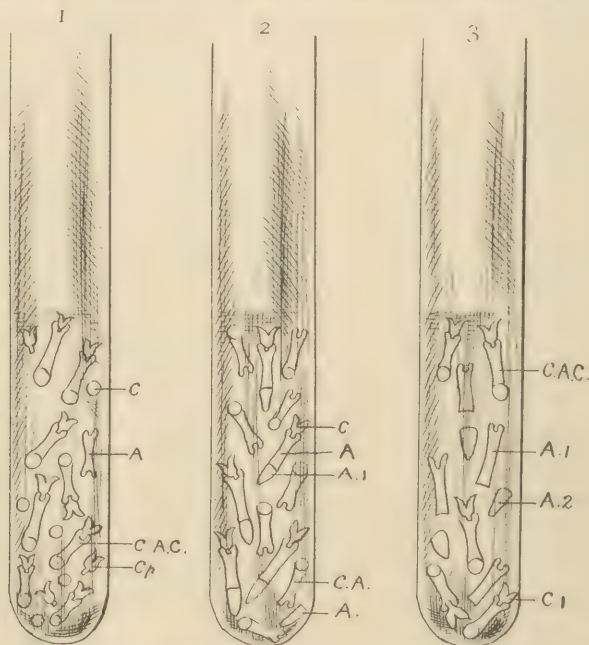


FIG. 117.—MECHANISM OF COMPLEMENT FIXATION.

Tube 1 shows the hemolytic system: *C*, a red blood-corpuscle; *A*, a hemolytic amboceptor; *C_p*, complement; *C.A.C.*, complement united to a corpuscle by means of the specific amboceptor. Hemolysis results.

Tube 2 shows complement fixation by bacterial antigen and amboceptor: *A₁*, antigen; *C*, complement united to the antigen *A₁* by the amboceptor *A*. When hemolytic amboceptors are added hemolysis does not occur because the complement has been previously fixed by the bacterial antigen and amboceptor.

Tube 3 shows absence of complement fixation because the bacterial amboceptor *A₁* is not specific for the bacterial antigen *A₂*, and hence complement is not fixed; when hemolytic amboceptor and the corresponding corpuscles are added complement unites with these, *C.A.C.*, and hemolysis occurs.

just described. As previously mentioned in a discussion of the mechanism of cytolysis, Ehrlich and his associates believed that complement and amboceptor may unite direct, which is in sharp contrast with the more generally accepted views of Bordet that complement or alexin is bound only by the antigen-sensitizer complex. Support for the theory of Ehrlich was supplied by Neisser and Wechsberg¹ who demonstrated that in bactericidal tests an excess of amboceptors may reduce the degree of bacteriolysis. They explained this phenomenon by stating that the amboceptors absorbed a portion of the free complement and thus prevented this portion from com-

¹ Münch. med. Wchn., 1901, 697.

binning with those amboceptors united with the bacteria, *i. e.*, the complement has been deviated or deflected from its natural course. In other words, that complement fixed by antigen and amboceptors may be said to be an example of complement fixation, whereas that fixed by amboceptors alone is an example of complement deviation.

There can be no doubt about the correctness of the fact that in bacteriolytic tests an excess of antiserum (amboceptors) may yield less bacteriolysis than smaller amounts of antiserum. This will be illustrated in the chapter on Bacteriolysins. Many investigators have corroborated these results. Furthermore, it is commonly encountered in quantitative complement-fixation tests, as the Wassermann test, employing varying amounts of serum. For example, the degree of complement fixation with 0.1 c.c. serum of a syphilitic may be less than occurs with 0.02 c.c.

But the explanation offered by Neisser and Wechsberg cannot be accepted. There is no experimental data to support the statement upon which it is based, namely, that complement and amboceptors unite direct. Rather all data supports the contention of Bordet that complement unites with amboceptors only when the latter have previously united with the antigen. Analogous phenomena are commonly observed in the agglutination and precipitin reactions when large amounts of immune sera produce less agglutination and precipitation than smaller amounts; these have been explained on the basis of the presence of agglutinoids and precipitoids. It is highly probable that these phenomena are colloidal reactions. Certainly the different constituents are of the nature of colloids and an excess of one colloid is known to inhibit the precipitation of a second, a reaction taking place only when the reacting bodies are present in definite proportions. Since this can be shown for agglutination and precipitation it is reasonable to assume that similar conditions are operative in complement deviation and that the phenomenon is one of colloidal chemistry.

Mechanism of Cytolysis or the Action of Complement (Alexin) and Amboceptor (Sensitizer).—With these general considerations regarding the amboceptors or sensitizers and the complements or alexins presented, we may now pass in review the opinions held regarding the mechanism of their action in the phenomenon of cytolysis. The more important amboceptors, as the bacteriolysins and hemolysins, will be taken up in more detail in subsequent chapters; likewise the properties of complement or alexin in special relation to complement fixation, but the mechanism of their action is regarded as identical and may be discussed at this time.

Baumgarten's Theory.—One of the earliest theories on the mechanism of bacteriolysis was that advanced by Baumgarten,¹ who taught that the solution of bacterial cells in specific serum was brought about by a change in osmotic conditions, that is, the antibodies in the serum uniting with the cell membranes rendered the bacteria permeable for salts and other substances which brought about swelling, increased intracellular pressure, and final destruction. This theory is now only of historical interest, and while salts play a rôle in the phenomenon of cytolysis, they do not occupy the important relation assigned them by Baumgarten as shown by the studies of von Lingelsheim² and others.

Buchner's Theory.—Buchner looked upon the bactericidal activity of normal and immune serum as due to one constituent, namely, alexin (protective substance), which he believed was of the nature of a proteolytic ferment because of its heat sensitiveness and instability on standing. Later

¹ Lehrbuch der pathogenen Mikroorgan., 1911.

² Ztschr. f. Hyg., 1901, 37.

researches by Bordet showed that this "alexin" was composed of two substances, namely, antibody and a normal constituent of serum. Bordet adapted the term "alexin" for this latter substance and named the former, or antibody, "sensitizer."

Ehrlich-Morgenroth Theory.—Following the discoveries of Nuttall, Buchner, Bordet, Pfeiffer, and other pioneer investigators in cytolysis, Ehrlich and Morgenroth¹ gradually evolved a theory of the mechanism of the phenomenon based largely upon the results of their own investigations and those of their associates by enlarging upon Ehrlich's side-chain theory to the third order of receptors. Briefly this is as follows:

The antigen (blood-corpuscles, bacteria or other cells) undergoes lysis when acted upon by two substances; one of these is the specific antibody, heat resistant, and produced during immunization, while the other is a non-specific constituent of serum, heat sensitive, and not increased by immunization as shown by Bordet² and von Dungern.³

To the antibody they gave the name "amboceptor," believing that it had two combining affinities or arms, namely, one for union with the cell, and the second for union with the second substance, to which they gave the name of "complement."

The specific antibody unites with its antigen direct even at 0° C. and regardless of the presence or absence of the complement; the antigen, however, shows no discernible changes, and in the case of living cells as bacteria, are not even killed. Complement must be present and united with the antigen-amboceptor complex before lysis can occur. Complement cannot unite with the antigen direct; it can do so only through the action of the interbody or amboceptor. The non-specific normal constituent of serum, namely, the ferment-like complement, is, therefore, the active lytic agent, but is powerless to dissolve the antigen until the latter is prepared by the specific amboceptors.

According to this theory complement may unite direct with the amboceptors without the antigen; this is how Neisser and Wechsberg explained their phenomenon of complement deviation, but, as previously stated, this direct union of complement and amboceptor lacks experimental proof.

Bordet's Theory.—Bordet, who analyzed Buchner's "alexin" into the two components of thermostabile and specific antibody and thermolabile or normal serum constituent, designated the antibody as "sensitizer" and the latter as "alexin." According to this theory⁴ the antibody unites with the antigen (blood-corpuscle, bacterial cell, etc.) direct, sensitizing or preparing it for the action of the alexin just as a mordant aids in the penetration of a dye in the staining of cells.

When antigen and antibody have united the antigen is regarded as "sensitized" or prepared for the lytic action of the alexin or complement. In the absence of the latter the sensitized antigen undergoes no appreciable changes.

Alexin or complement cannot unite direct to the antigen; union can only occur with sensitized or "antibodyized" antigen.

Alexin or complement cannot unite direct with the antibody or sensitizer; in this respect the theory of Bordet takes sharp issue with the theory of Ehrlich. This will be discussed a little later in connection with the subject of conglutinins.

¹ Studies in Immunity, Ehrlich-Bolduan, 1910.

² Ann. d. l'Inst. Pasteur, 1898, 12, 688.

³ Münch. med. Wchn., 1900, No. 20.

⁴ Studies in Immunity, Bordet-Gay, 1909.

Metchnikoff's Theory.—Metchnikoff¹ admits the production of the specific antibody (Bordet's sensitizer or Ehrlich's amboceptor), and has called it "fixator." He asserted that the amount of antibody produced is proportional to the degree of phagocytosis and phagolysis that occur during the absorption of the antigen. In other words, he has sought to maintain the importance of his theory of phagocytosis by asserting that the antibody is a product of phagocytic cells, its production running parallel with the degree of leukocytosis, and injury during the process of immunization. Metchnikoff considers the "fixators" as analogous to enterokinase, and he believes that, like the latter, the fixation acts as an accessory digestive ferment, having for its object the linking of the more potent ferment, as a cytase (alexin or complement), to the cell (antigen) to be digested (lysis).

Conglutinins in Relation to Theories of Cytolysis; Auxilysins.—As stated above, the views of Bordet and Ehrlich differ in regard to the power of alexin or complement for union direct to free sensitizer or amboceptor. As proof of their belief that this union may occur Ehrlich and Sachs² have described their "Experimentum Crucis" as follows:

(a) Fresh horse-serum + guinea-pig corpuscles gives slight hemolysis.

(b) Fresh horse-serum + heated beef-serum + guinea-pig corpuscles gives marked hemolysis. Presumably this is due to the presence of anti-guinea-pig hemolytic amboceptor or sensitizer in the beef-serum; but if the corpuscles and heated beef-serum are first mixed and centrifuged the corpuscles do not hemolyze upon the addition of complement, that is, are not sensitized. Ehrlich and Sachs argued that this showed that the amboceptor in the beef-serum unites first with the complement of the horse-serum independently and that this complex then unites with the corpuscles and results in hemolysis. If guinea-pig amboceptor is present in the beef-serum and fails to be removed when the serum is treated with these cells, it would also tend to prove that both Ehrlich's and Bordet's views on sensitization or direct union of antigen and antibody were incorrect, but further investigations by Bordet and Gay³ have shown that this is a peculiar phenomenon to be separated from the mechanism of hemolysis in general.

They found that guinea-pig cells are hemolyzed very slowly by guinea-pig complement or alexin and antiguinea amboceptor or sensitizer; however, if heated beef-serum was added agglutination and hemolysis were very rapid. They, therefore, advanced the hypothesis that the complex of cells + antibody + alexin (complement) attracts a colloidal substance from beef-serum which unites with them and produces two results: Powerful agglutination followed by hemolysis; plain cells or cells + antibody cannot attract it.

Bordet and Gay named this substance in beef-serum "bovine colloid." Bordet and Streng⁴ later suggested the name *conglutinin*. Streng⁵ later showed the presence of conglutinins in beef-serum for bacteria and also that conglutinins were present in the sera of goats, sheep, antelopes, and other herbivora, but not in the sera of cats, dogs, guinea-pigs, and birds. A further discussion of this substance and the technic of the conglutination test are given in the chapter on Agglutinins.

Manwaring⁶ has found that normal goat-serum heated to 56° C. for three or four hours acquires the property of increasing the hemolytic and

¹ See Immunity and Infective Diseases, 1905.

² Berl. klin. Wchn., 1902, No. 21.

³ Ann. d. l'Inst. Pasteur, 1906, 20, 467.

⁴ Centralbl. f. Bakteriöl., 1909, 49.

⁵ Ztschr. f. Immunitätsf., 1909, 2, 415.

⁶ Centralbl. f. Bakteriöl., 1906, 42, 75.

hemagglutinative properties of goat antishoop hemolysin. He has called these substances in the heated goat-serum *auxilysins* (to increase), and apparently these are similar to the conglutinins described above.

RÔLE OF CYTOLYSINS IN IMMUNITY

The very important relation of certain of the cytolytins, notably those for pathogenic parasites of both vegetable and animal origin to resistance to disease and recovery from infection, is generally conceded. In general terms it may be said that all phases of immunity may be resolved into the activities of phagocytes and opsonins, antitoxins and cytolytins, the agglutinins and precipitins being accessory in their activities to the cytolytins.

The bacteriolysins found normally in the blood are doubtless one important means of natural immunity to certain micro-organisms, and in infections are greatly increased, particularly in diseases due to pathogenic bacilli. The complements apparently play an important rôle in physiologic processes of metabolism and repair, as also in the processes of natural immunity and recovery from disease. While no means has been discovered for bringing about their increase by processes of immunization, their production is known to fluctuate during health and disease, and probably as demands are made for cytolytic processes.

A complete discussion of these cytolytins in relation to immunity is given in Chapter XXVI; the rôle of some of the more important members of the group, as the bacteriolysins, hemolysins, and cytotoxins, is discussed in the separate chapters devoted to these antibodies.

CHAPTER XIX

BACTERIOLYSINS

HAVING considered the general nature and properties of amboceptors and complements and the mechanism of their action in producing solution or lysis of cells, we will now study more closely the *bacteriolysins*, which are antibodies belonging to this group and possessing diagnostic and considerable therapeutic importance.

Historic; the Bactericidal Activity of Normal Blood and Serum.—The early history of the discovery of the bacteriolysins is closely associated with the history of immunity in general, for with the discoveries in bacteriology, and the establishment of the relation of bacteria to disease, it followed as a matter of course that investigations should be undertaken to ascertain the mechanism of resistance to and of recovery from an infection.

In 1874 Traube and Gscheidlen¹ showed that septic material may be destroyed in the blood of living animals, and in 1881 Lister demonstrated the same phenomenon in extravascular blood. These experiments were naturally somewhat crude, as they antedated the period in which the pyogenic micro-organisms were isolated and studied in pure culture, but they served, nevertheless, to demonstrate the germicidal powers of the blood.

In 1886 Fodor² demonstrated the germicidal action of the defibrinated blood of the rabbit upon anthrax bacilli. This work was followed shortly after by that of Flügge³ and Nuttall,⁴ who showed the germicidal powers of the body fluids in general independent of cells. Nuttall was able to follow step by step the destruction of anthrax bacilli on the warm stage of the microscope by defibrinated rabbit blood. The same phenomenon was shown by plating methods. Nuttall also discovered that when blood was heated to 55° C. the bactericidal properties were completely destroyed. These observations gave Flügge and his assistant Bitter the opportunity to criticize vigorously the theory of phagocytosis, and the controversy between the adherents of the cellular and humoral theories of immunity now began, as Metchnikoff was actively engaged in studying phagocytes and in formulating his phagocytic theory.

Buchner⁵ and others took up the subject emphasizing the important germicidal powers of the body fluids and ascribing this function to the presence of "alexins" (substances that ward off disease). Buchner also found that if the serum was heated this germicidal power was lost; hence it followed that the active bacteriolytic agent was considered very unstable and was quickly destroyed outside of the body. He was able without difficulty to confirm Nuttall's discoveries and added to them new facts of great value.

In 1894 Pfeiffer⁶ demonstrated most clearly the phenomenon of bacteriolysis which gave great encouragement and impetus to studies in immunity, and incidentally strengthened the claims of the humoral theory.

¹ Jahresb. d. schles. Gesellsch. f. vaterl. Kult., Breslau, 1874.

² Deutsch. med. Wchn., 1886, 617; *ibid.*, 1887, 745.

³ Ztschr. f. Hyg., 1888, 4, 208, 223.

⁴ Ztschr. f. Hyg., 1888, 4, 253.

⁵ Arch. f. Hyg., 1890, 10, 84; Centralbl. f. Bakteriöl., 1889, 5, 817; *ibid.*, 6, 561; 1890, 8, 65.

⁶ Ztschr. f. Hyg., 1894, 16, 268; *ibid.*, 1894, 18, 1.

He showed that cholera vibrios introduced into the peritoneal cavity of a guinea-pig that had been immunized against cholera lost their motility and finally became disintegrated and passed into solution regardless of the presence of cells.

As stated in the preceding chapter, it remained for Bordet, however, to show the mechanism of this interesting phenomenon. This observer demonstrated the fact that the thermolabile body was but one substance concerned in the reaction, and that the specific substance was thermostable and the actual product of immunization, results that were later corroborated and elucidated by his researches upon hemolysis, and by those of Ehrlich and his pupils on cytolytic phenomena in general. As previously mentioned, Bordet retained the name "alexin" for the thermolabile substance and applied the new term, "substance sensibilisatrice," to the specific thermostable antibody. Later both substances were renamed by Ehrlich, and called "complement" and "amboceptor" respectively.

As will be pointed out further on, as the result of these observations Metchnikoff modified his phagocytic theory, and recognized the existence of both substances, which he named "cytases" and "fixateurs," believing that both were derived from cells classed as phagocytes.

All are agreed as to the presence of two different bodies in the body fluids concerned in bacteriolysis, although opinions vary as regards their origin and mechanism of action. The side-chain theory has been widely accepted in explanation of their action, and the terms applied by Ehrlich to the two substances concerned, namely, complements and amboceptors, are in general use.

Definition.—*Bacteriolysins are substances present in the serum and other body fluids that kill bacteria with or without lysis.*

The term itself would infer that solution or lysis of the bacterium is an essential property of an antibody of this order. *Bactericidins* are substances that kill bacteria without lysis, and, strictly speaking, an effort should be made to differentiate between the terms, although from a practical standpoint this is not important. Certain micro-organisms may be killed and resist solution or digestion for a comparatively long time, whereas, on the other hand, the same bacteria, under different circumstances, may readily be lysed.

Although the endotoxins liberated from the lysed bacteria may produce symptoms of disease, followed by death, yet the bacterium itself is usually destroyed and unable to proliferate. A bacteriolysin is, therefore, always bactericidal, although the converse is not necessarily true.

Custom, however, has never strictly differentiated between the two terms, and bacteriolysis appears to be but a continuation of and a more nearly complete bactericidal process. Hence the definition just given covers both terms—bacteriolysins and bactericidins.

Origin of Bacteriolysins.—Our knowledge regarding the origin of the bacteriolysins is quite fragmentary and has already been discussed in the preceding chapter. The investigations of Pfeiffer and Marx in cholera, and Wassermann in typhoid, have shown that the spleen and hematopoietic organs in general may be especially concerned.

According to Metchnikoff the "bacterial fixateurs," which are practically the bacteriolysins, are secretory or excretory products of phagocytic cells, especially the polynuclear leukocytes or microphages. As early as 1889 he¹ expressed the opinion that a portion at least of the bactericidal power might come from substances that had escaped from the leukocytes

¹ Ann. de l'Inst. Pasteur, 1888, 3, 670.

during the preparation of defibrinated blood and of serum. It is commonly believed that during infection the bacteria cause phagolysis or disintegration of these cells, with liberation of both complements (cytases) and amboceptors (fixateurs), which produce extracellular lysis of the invading bacterium (bacteriolysis). If, on the other hand, the phagocytes are fortified and phagolysis is prevented, the bacteria are phagocytized and undergo intracellular lysis, a condition that, according to Metchnikoff, may be induced experimentally by giving an animal an intraperitoneal injection of sterile bouillon twenty-four hours before bacteria or other cells are injected.

Leukocidins and Leukocytic Extracts.—That leukocytes afford a bacteriolytic substance is supported by observations showing that leukocytic exudates, secured by the injection of a sterile aleuronat suspension, possess a well-marked germicidal activity. Denys and Havet¹ were the first to show that exudations rich in leukocytes exhibited a bactericidal power much higher than that of the corresponding sera. Shortly afterward Buchner² observed the same on comparing the bactericidal power of exudations rich in leukocytes with the blood-serum of the same animals. Issæff³ found that the intraperitoneal injection of sterile bouillon and other mild irritants, by producing a leukocytic exudate that supplied certain bactericidal substances and facilitated phagocytosis, increased the resistance of animals to bacterial infection. At one time surgeons made practical use of this observation by injecting nucleinic acid and other substances into the peritoneal cavity before performing laparotomy in order to induce a local resistance to a possible infection.

The bactericidal substance contained within leukocytes has been extensively studied by Schattenfroh,⁴ Petterson,⁵ Hiss,⁶ Kling,⁷ and others. It has been observed that when leukocytes are suspended in diluted blood-serum the bactericidal properties of the serum are increased without coincident destruction of the cells, showing that the leukocytes may secrete germicidal substances into the fluid. The same observation has been made with Bier's congestive lymph, indicating that this activity takes place both in the test-tube and in living tissues.

Watabiki⁸ has questioned the bactericidal activity of the endolysins or leukins of leukocytes. He could find no evidence of bactericidal activity of extracts of rabbit leukocytes for such bacteria as typhoid, cholera, colon, and dysentery bacilli, nor in extracts of spleen, liver, and bone-marrow of normal rabbits. Manwaring⁹ found that the active antibacterial substance of leukocytes resembled certain enzymes which can be isolated and purified by alcoholic precipitation. This phase of the subject is considered more fully in Chapter XXXIX.

Hiss¹⁰ and, later, Hiss and Zinsser¹¹ found that autolyzed leukocytic exudates possess some bactericidal activity, and that they may profoundly modify experimentally induced infection of rabbits and guinea-pigs with the pneumococcus, staphylococcus, streptococcus, and other bacteria. In applying this method of treatment to man by means of subcutaneous

¹ La Cellule, 1894, 10, 7.

² Münch. med. Wchn., 1894, 717.

³ Ztschr. f. Hyg., 1894, 16, 287.

⁴ Arch. f. Hyg., 1897, 28, 135.

⁵ Ztschr. f. Immunitätsf., 1908, 1, 52; *ibid.*, 1910, 7, 693.

⁶ Jour. Med. Research, 1908, 19, 323.

⁷ Ztschr. f. Immunitätsf., 1910, 7, 1.

⁸ Jour. Infect. Dis., 1909, 6, 319.

⁹ Jour. Exper. Med., 1912, 16, 249.

¹⁰ Jour. Med. Res., 1909, 20, 245.

¹¹ Jour. Med. Res., 1910, 22, 397.

injections these investigators observed distinctly beneficial results in cases of epidemic cerebrospinal meningitis, lobar pneumonia, staphylococcus infections, and erysipelas (see Chapter XL).

Gengou¹ has recently reviewed this subject, and studied the relationship of these leukocytic bacteriolysins to alexin (complement). He found that a solution of washed polymorphonuclear leukocytes, made by dissolving the cells in centinormal HCl and subsequently neutralizing the solution, has active bacteriolytic properties. Gengou thinks that this extract contains the substances which dissolve bacteria in the interior of the leukocytes after phagocytosis. The properties of these substances extracted from leukocytes were as follows:

1. The extract is bacteriolytic for a number of different bacteria, showing no specificity.
2. The range of temperature through which the substances act is very broad, from 20° to 60° C. These substances are quite resistant to heat.
3. The bacteriolytic substances from leukocytes retain their activity in neutral and acid fluids for many days.
4. The extracts, while energetically bacteriolytic, are not hemolytic, and they do not cause hemolysis of red cells sensitized with amboceptor. As the addition separately of the end-piece and mid-piece of complement does not cause the extract to take any part in the specific hemolytic reaction, it is concluded that the extract lacks both those parts of complement.
5. These substances are not simply activated by the acid used to dissolve the cells. They are themselves dissolved in an acid solution at first, but later cause bacteriolysis only in a neutral fluid.
6. These extracts are proteolytic, in that they liquefy gelatin. The proteolytic properties, however, are less resistant to heat than the bacteriolytic, and act best at different ranges of temperatures. Some actively bacteriolytic extracts are devoid of demonstrable proteolytic action.
7. The characteristic effect of these extracts upon bacteria is a solution or granular disintegration of the micro-organisms.

Gengou concludes that the alexin or complement of serum is different from these bacteriolytic substances which he has extracted from leukocytes. While he has found that these endolysins were non-hemolytic, Carrel and Ebeling,² on the other hand, found that cultures of leukocytes *in vitro* in hen serum sometimes elaborated a substance hemolytic for sheep and rabbit corpuscles, whether this substance was an alexin, a sensitizer, or a complex sensitizer alexin, as described by Hyde,³ was not determined. At any rate it is fairly well established that leukocytes are one source of hemolysins as well as of other cytolsins; this subject will be discussed in more detail in the following chapter. The experiments of Carrel and Ebeling also showed that leukocytes, cultivated in plasma, always secrete substances which increase the rate of growth of homologous cells. This indicates the importance of these cells, not only in relation to processes of resistance and immunity to infection, but to repair of injured tissues as well. Substances of a bacteriolytic nature are produced not only by leukocytes, but probably by other body cells as well, which are to be found not only in the blood but in the secretions and tissues, as indicated by the recent investigations of Fleming and Allison.⁴

Preparation of Leukocytic Extracts.—Hiss and Zinsser have prepared

¹ Ann. d. l'Inst. Pasteur, 1921, 35, 497.

² Jour. Exper. Med., 1922, 36, 645.

³ Amer. Jour. Hyg., 1921, 1, 358.

⁴ Jour. Exper. Path., 1922, 3, 252.

leukocytic extract by giving rabbits intrapleural injections of aleuronat suspension. Manwaring has secured much larger quantities by making his injections into the horse.

The aleuronat is prepared by making a 3 per cent. solution of starch in bouillon without heating, and adding 5 per cent. of powdered aleuronat to this emulsion. The starch helps to keep the aleuronat in suspension. The mixture is boiled for five minutes and placed in large sterile test-tubes, 20 c.c. being placed in each tube. Final sterilization is done in an autoclave.

For making the injections large rabbits are selected. The hair over both sides of the thorax is removed, the skin is sterilized with tincture of iodine, and 10 c.c. of the aleuronat suspension are injected into each pleural cavity at a point in the anterior axillary line, at the level of the sternum, great care being taken to avoid puncturing the lungs. After twenty-four hours the animals are chloroformed and the pleural cavities carefully and aseptically opened. The cellular exudate is pipetted into centrifuge tubes containing at least 10 c.c. of sterile 1 per cent. sodium citrate in normal salt solution. One or more cubic centimeters of exudate may be obtained from each cavity. The exudate is usually tinged with blood. It is then centrifuged and the supernatant fluid removed. The sediment is broken up with a platinum spatula, and 20 volumes of sterile distilled water are added. The tubes are set aside in the incubator for twenty-four hours, after which cultures are made to insure sterility. A small amount of preservative may be added, and the extract placed in bottles or ampules ready for administration. It is given subcutaneously in doses of from 5 to 10 c.c. every four to six hours.

Archibald and Moore¹ have described a method of securing leukocytes from the circulating blood of normal animals. This consists of bleeding the horse, dog, or other domestic animal from a jugular vein into sterile flasks containing a sufficient quantity of 1 per cent. sodium citrate solution to prevent coagulation. To this suspension is added 0.5 per cent. acetic acid and the mixture centrifuged. The sediment is washed several times with saline solution, ground with sterile sand, and neutralized if necessary. To the resulting product is added four volumes of sterile water followed by heating at 58° C. for one hour, after which it is placed in the incubator until digestion is complete. The material is again centrifuged and the supernatant fluid employed preserved with tricresol.

The endocellular bactericidal substances, or *endolysins*, mentioned in a previous chapter, which can be extracted from leukocytes, are not in the nature of complements, as they are not rendered inactive by temperatures below 80° C. They cannot apparently be increased by immunization, the quantity present in each leukocyte being probably at all times just sufficient for the digestion of a limited number of bacteria, which can be ingested at one time by the individual leukocyte. It is probable that the excess of bactericidal substance is thrown off into the blood-stream, representing the serum bacteriolysins, and at least indicating that the leukocytes are one source for the production of bacteriolytic amboceptors.

Bactericidal Activity of Whole Blood Versus Plasma and Serum.—The maximum degree of bactericidal activity is observed with whole blood. Striking evidence of this has been recently supplied by the investigations of Heist, Cohen and Cohen,² who showed that the natural immunity of the pigeon to virulent pneumococci was due in large part to the bactericidal activity of the whole blood. Rabbit and mouse bloods were found prac-

¹ Jour. Exper. Med., 1912, 16, 249.

² Jour. Immunology, 1918, 3, 261.

tically without bactericidal activity, and the high susceptibility of these animals to the pneumococcus is well known. Matsunami and myself¹ observed similar results in respect to natural immunity to virulent meningococci. Matsunami² has likewise observed that with the Heist-Lacey bactericidal test whole coagulable blood was more active than defibrinated and citrated blood and serum, probably because phagocytosis was operative and aided in the former. Halm³ has reported a hyston plasma just as bactericidal as serum, but Metchnikoff has maintained, largely on the basis of the experiments of Gengou,⁴ that plasma is without bactericidal activities, whereas serum is bactericidal due to the presence of substances derived from leukocytes during the process of coagulation. Watanabe,⁵ in my laboratory, found plasma to contain about as much complement and antibody as serum.

Mechanism of Bacteriolysis.—According to the side-chain theory of Ehrlich a bacteriolysin is an antibody of the third order, or an amboceptor furnished with two haptophore or grasping arms for uniting the bacterium on one side with a suitable complement on the other. The antibody, therefore, acts simply as an interbody or connecting link; it is specific for the bacterium causing its production, but is unable itself directly to injure the bacterium, lysis being brought about by an attached complement. Bacteriolysis is, therefore, an interaction of amboceptor and complement upon the bacterial body (Fig. 118).

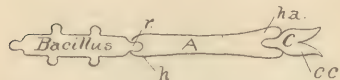


FIG. 118.—THEORETIC STRUCTURE OF A BACTERIOLYTIC AMBOCEPTOR.

A, Amboceptor; C, complement; r, receptor of bacillus; h, haptophore group of the amboceptor; h.a., complementophile group of the amboceptor.

While bacteriolytic amboceptors will unite with their antigens under practically all conditions, nevertheless a suitable complement may not be present, and hence a bacteriolytic serum may not be active in all animals.

The influence of bacteriolysins upon endotoxins is a question of considerable interest and importance. As the result of convincing experiments performed, especially by Pfeiffer, it is evident that a bacteriolytic serum does not neutralize the endotoxin at the time the bacterium undergoes disintegration. Highly immune serums appear to be unable to protect an animal against the endotoxins, and, indeed, may even increase the intoxication, and, by liberating an excess of endotoxin, kill the animal.

The bactericidal substances derived from leukocytes are, however, apparently capable of neutralizing endotoxins, to some extent at least, as Hiss and Zinsser were unable to ascribe the beneficial effects of leukocytic extracts to the bacteriolytic action alone.

As mentioned elsewhere, both Metchnikoff and Bordet maintain that the bacteriolysin is in the nature of a "sensitizer," preparing the bacterium for the action of the alexin or cytase, just as a mordant aids in the penetration of a dye-stuff.

Bactericidal Activity Without Complement.—As stated above, complement or alexin is ordinarily required for the phenomenon of bacteriolysis. Therefore, it is generally true that the antibacterial properties of both normal and immune sera are greater when perfectly fresh than after being heated or allowed to stand for weeks or months with the addition of a preservative, which permits the complete destruction of complement.

¹ Jour. Immunology, 1918, 3, 201.

² Jour. Immunology, 1920, 5, 51.

³ Berl. klin. Wchn., 1896, 864.

⁴ Ann. d. l'Inst. Pasteur, 1901, 15, 232.

⁵ Jour. Immunology, 1919, 4, 77.

Normal human serum, however, often shows some bactericidal activity even after heating to 55° C., as shown by Selter¹ and others. The nature of this thermostabile bactericidal substance is unknown. Flexner,² as likewise Matsunami and myself,³ have observed the presence of these bactericidal substances in horse antimeningococcus sera kept many months and frequently heated and absolutely free of complement. Black, Fowler, and Pierce⁴ have also reported that heating typhoid and dysentery antisera did not materially reduce their bactericidal activity when tested by the Heist-Lacy method. I believe these bactericidal substances are derived from the leukocytes, being the bactericidal leukins or endolysins of these cells, which are known to be thermostabile and active in the absence of complement.

General Properties of Bacteriolysins.—As with other cytolytins, the bacteriolysins are thermostabile and resist heating to 60° C., being gradually destroyed at temperatures ranging from 70° to 80° C. They are likewise highly resistant to acids and alkalies, and when preserved in a sterile condition with the addition of small quantities of a preservative bacteriolytic serums for therapeutic and diagnostic purposes may remain active for long periods of time. Cholera immune serum as a diagnostic aid in making the agglutination and Pfeiffer bacteriolytic reactions is best preserved in dry form, the serum retaining its activity under these conditions for considerable periods of time.

Natural Bacteriolysins.—As a result of the contention of Metchnikoff that bacteriolysins (fixateurs) are produced only upon the disintegration of leukocytes, and that the plasma is accordingly free from these antibodies, much experimental work has been done. The weight of evidence is against this view, as both amboceptors and complements have been demonstrated in plasma, although the quantity of bacterial amboceptors normally present in the body fluids is quite small. It is quite natural to expect that under normal conditions small amounts will be present, as receptors are being constantly thrown off into the blood, and leukocytes are, of course, being constantly formed and destroyed.

The amounts of natural bacteriolysins in human blood and that of the lower animals vary greatly for different bacteria. For example, Nissen⁵ showed many years ago that while normal rabbit blood was highly bactericidal for the anthrax bacillus, as demonstrated by Fodor and Nuttall, bactericidal activity varied greatly for other bacteria, being practically absent for staphylococci and streptococci and most active for the pneumococcus and bacilli of the typhoid-colon-cholera-dysentery group.

Meltzer and Norris⁶ found that prolonged fasting had no influence upon the bactericidal activity of normal dog-serum upon typhoid bacilli, although Canalis and Morpurgo⁷ claim that starvation removes or diminishes the natural immunity of the pigeon to anthrax bacilli.

As previously stated, the bactericidal power of defibrinated blood and fresh serum is due to the presence of complement (alexin) and natural antibody (bacteriolysin). After heating to 55° C. for ten to thirty minutes bactericidal activity is frequently (not always) lost, due to the destruction of complement. The presence of natural bacteriolysins may then be demonstrated by treating the serum with bacteria, removing them after allowing a suitable period for union with the bacteriolysin by centrifuging, and

¹ Ztschr. f. Hyg., 1918, 86, 313.

² Jour. Exper. Med., 1908, 10, 141.

³ Jour. Immunology, 1918, 3, 157.

⁴ Jour. Amer. Med. Assoc., 1920, 75, 915.

⁵ Ztschr. f. Hyg., 1888, 4, 353.

⁶ Jour. Exper. Med., 1899, 4, 131.

⁷ Fortschr. d. Med., 1890, 8, 693, 729.

treating the sediment with guinea-pig serum as complement. Of course, the complement serum must be free of natural bacteriolysin for the bacteria under study in order to avoid erroneous results.

The blood of man and many of the lower animals contains appreciable amounts of bacteriolysins for many different bacteria. As recently indicated by the investigations of Heist, Cohen and Cohen,¹ Kolmer and Matsunami,² Black, Fowler and Pierce,³ and others many examples of natural and acquired immunity are to be ascribed to these bactericidal agents.

Specificity of Bacteriolysins.—The bacteriolysins are highly specific antibodies, and are useful in making the differentiation of bacterial species. Group bacteriolytic reactions are less common as compared to group agglutination, as was shown by Kolmer, Williams, and Raiziss⁴ with the typhoid colon group of bacilli. As a practical procedure, however, the agglutination and complement-fixation reactions are so easily secured as to be the tests of choice in making a differentiation between closely allied organisms. As with the agglutinins, the influence of partial bacteriolysins may be removed by using highly immune serums in high dilutions.

Immune Bacteriolysins.—These are produced during disease and by means of artificial immunization. Different bacteria vary greatly in the amount of bacteriolysins they are capable of inducing the body cells to produce. For example, the pathogenic cocci (staphylococci, streptococci, and pneumococci) induce the production of relatively small amounts of bacteriolysin and large amounts of opsonin, whereas the bacilli of typhoid-colon-dysentery-cholera group induce the production of large amounts of bacteriolysins as shown by the investigations of Stern and Korte,⁵ Laubenthaler,⁶ Korte and Steinberg,⁷ Topfer and Jaffe,⁸ Neufeld and Hune,⁹ Wright,¹⁰ Sluga,¹¹ Richardson,¹² and others. Torrey¹³ has shown that normal and immune rabbit sera may be highly bactericidal for gonococci.

Rôle of Bacteriolysins in Immunity.—Doubtless the natural and immune bacteriolysins and bactericidans exert a very important and fundamental influence upon natural and acquired resistance and recovery from bacterial infections. This is especially true of infections with pneumococci, meningococci, and bacilli of the typhoid-colon-cholera-dysentery group and is discussed in more detail in subsequent chapters.

All of the immune sera employed for the treatment of bacterial infections contain some of the bacteriolysins which exert an important influence upon their curative properties; this is especially true of antipneumococcus, antimeningococcus, and antidysentery sera, and of lesser importance with diphtheria and tetanus antitoxic sera. This subject is discussed in more detail in those chapters dealing with the various immune sera employed in the treatment of disease.

Practical Applications.—The bacteriolysins have considerable value in the following procedures:

1. In making a differentiation of bacteria, especially when the presence of cholera vibrios is suspected.
2. In the diagnosis of certain infections, such as cholera and typhoid fever.
3. In the treatment of some infections with specific bacteriolytic serums.

¹ Jour. Immunology, 1918, 3, 261.

⁸ Ztschr. f. Hyg., 1906, lli, 393.

² Jour. Immunology, 1918, 3, 177.

⁹ Arb. a. d. Gsndhtsamte, 1907, 25, 16.

³ Jour. Amer. Med. Assoc., 1920, 75, 915.

¹⁰ Lancet, 1901, 609.

⁴ Jour. Infect. Dis., 1913, 13, 321.

¹¹ Berl. klin. Wchn., 1904, xli, 79.

⁵ Berl. klin. Wchn., 1904, xli, 213.

¹² Jour. Med. Research, 1901, 6, 187.

⁶ Ztschr. f. klin. Med., 1905, lvi, 170.

¹³ Jour. Med. Res., 1908, 19, 471.

⁷ Deut. Arch. f. klin. Med., 1905, lxxxii, 321.

TECHNIC OF BACTERIOLYTIC TESTS

THE PFEIFFER EXPERIMENTS

The essentials of this important test have been described at the beginning of this chapter. Briefly, it consists in making intraperitoneal injections of a bacteriolytic serum mixed with living bacteria into a normal guinea-pig. The resulting bacteriolysis is studied microscopically by withdrawing small amounts of peritoneal exudate at varying intervals. By performing the experiment with varying dilutions of serum the bacteriolytic titer may be determined by noting the dilution in which bacteriolysis just fails to occur in a specified time.

Pfeiffer also showed that the phenomenon could be produced by injecting a mixture of serum from an immunized animal and the culture of cholera into the peritoneum of a normal guinea-pig. This phenomenon appeared when an old specimen of serum was used as well as when a fresh specimen that had been heated to 60° C. was employed. Later this observer found that if an old immune serum was injected into the peritoneal cavity and allowed to remain for a time, it regained its bactericidal powers.

Pfeiffer believed that the bacteriolytic substance may exist in the serum of an immunized animal either in an active or in an inert state. In the blood-serum or peritoneal fluid of the living animal it occurs as an active substance, but when kept for a few days or when heated rapidly to 60° C. it becomes inert; it may be rendered active again by coming in contact with the lining endothelial cells of the peritoneum.

The foregoing constitutes the classic Pfeiffer experiment. The bacteriolytic amboceptor present in the immune serum is activated by the complement furnished by the guinea-pig. The same serum will not produce bacteriolysis in the test-tube in case it has been heated or the complement is lost through age unless fresh normal serum or peritoneal exudate is added. By immunizing guinea-pigs with gradually increasing doses of cholera serum and then introducing fatal doses of cholera culture intraperitoneally, the same phenomenon of bacteriolysis is observed. In this instance the guinea-pig furnishes both amboceptor and complement.

By these and similar experiments and observations Bordet was able to show the rôle played by the two bodies concerned in cytolysis in general, namely, the thermolabile alexin or complement and the specific sensitizer or bacteriolytic amboceptor.

Bacteriolytic Test *in Vivo* for the Identification of Bacteria Recovered from Feces, Water-supplies, etc.—This method is employed chiefly in the identification of suspected cholera cultures. According to Citron, in Germany, the Pfeiffer test, made with micro-organisms obtained in pure culture from suspected patients is required for the official diagnosis of the first cases of cholera.

As a rule, the agglutination test is first applied in making the diagnosis of a suspected micro-organism, as the technic of this test is more easily carried out.

This bacteriolytic test may also be employed in the study of typhoid and paratyphoid bacilli, although bacteriolysis of these micro-organisms is less complete than that observed with cholera, and agglutination tests answer all practical requirements.

The test consists in mixing varying dilutions of a known and highly immune serum with a constant dose of unknown micro-organisms, and injecting the mixtures intraperitoneally into guinea-pigs. After twenty minutes small amounts of exudate are withdrawn by means of fine capil-

lary pipets, and studied in hanging-drop preparations. In the presence of a positive reaction the bacilli are observed to lose motility, become swollen and coccoid in shape, and gradually form granules, ultimately disappearing in complete solution.

Preparing the Immune Serum.—A highly immune serum is required. This may readily be prepared by giving rabbits a series of intravenous injections of a known culture, according to the technic described for the preparation of bacterial agglutinins. The official test in Germany demands that the serum be of such strength that 0.0002 gm. of dried serum will suffice to disintegrate completely within one hour 1 loopful (2 mg.) of an eighteen-hour-old culture of virulent cholera in 1 c.c. of nutrient bouillon when injected into the peritoneal cavity of a guinea-pig. The Hygienic Laboratory in Washington is prepared to furnish board of health laboratories with a dried serum of high titer for diagnostic purposes.

In testing an immune serum to determine its bacteriolytic titer the dose of micro-organisms should not be larger than 1 loopful, so that if any particular strain of cholera or typhoid is not sufficiently virulent, necessitating the use of larger doses, the virulence should be increased by passing the organism through guinea-pigs.

Method of Testing the Virulence of a Culture.—The unit of measurement is a 2 mm. platinum loop, which, when loaded, will usually hold about 2 mg. of micro-organisms. All dilutions are made with sterile neutral broth, and not with salt solution. Mixtures are injected intraperitoneally into 250-gm. guinea-pigs to determine the dose that will be fatal within twenty-four hours.

Great care should be exercised in all manipulations to avoid accidental infection. The mouth-ends of pipets should be plugged with cotton. Sufficient assistants should be on hand to facilitate the making of injections and the examination of peritoneal exudates with ease, caution, and certainty. All pipets, measuring glasses, test-tubes, and hanging-drop preparations should be immersed after using in 1 per cent. formalin solution before cleaning. In other words, every precaution should be taken to carry out a thorough and conscientious bacteriologic technic.

Guinea-pig No. 1: 4 loopfuls of agar culture emulsified in 4 c.c. of bouillon; inject 1 c.c. intraperitoneally (= 1 loopful).

Guinea-pig No. 2: 1 c.c. of foregoing emulsion + 1 c.c. of bouillon; inject 1 c.c. intraperitoneally (= $\frac{1}{2}$ loopful).

Guinea-pig No. 3: 1 c.c. of first emulsion + 4 c.c. of bouillon; inject 1 c.c. intraperitoneally (= $\frac{1}{3}$ loopful).

Guinea-pig No. 4: 1 c.c. of emulsion No. 3 + 1 c.c. of bouillon; inject 1 c.c. intraperitoneally (= $\frac{1}{10}$ loopful).

A satisfactory culture is one in which a dose of $\frac{1}{3}$ loopful will prove fatal within twenty-four hours. The immune serum is then titrated with five times this amount of culture, or 1 loopful.

Method of Titrating a Bacteriolytic Serum.—The serum is inactivated by heating to 56° C. for half an hour, and dilutions are made with bouillon in sterile shallow glasses or test-tubes. One loopful of an eighteen-hour agar culture of the micro-organism is thoroughly emulsified in the diluted serum, and the mixtures are injected intraperitoneally in 250-gm. guinea-pigs. Higher dilutions than those given here may be employed until the limit of bacteriolytic activity is reached.

1. Mix 0.5 c.c. of inactivated serum with 4.5 c.c. of bouillon (1 : 10). Place 2 c.c. of this mixture in a separate test-tube and add 2 loopfuls of culture. Inject 1 c.c. (= 0.1 c.c. immune serum).

2. Mix 2 c.c. of the first dilution (1 : 10) with 18 c.c. of bouillon (= 1 : 100). Place 2 c.c. in a separate tube and add 2 loopfuls of culture. Inject 1 c.c. (= 0.01 c.c. immune serum).

3. Mix 1 c.c. of the second dilution (1 : 100) with 4 c.c. of bouillon (= 1 : 500). Place 2 c.c. in a separate tube and add 2 loopfuls of culture. Inject 1 c.c. (= 0.05 c.c. immune serum).

4. Mix 2 c.c. of the third dilution (1 : 500) with 2 c.c. of bouillon (= 1 : 1000). Place 2 c.c. in a separate tube and add 2 loopfuls of culture. Inject 1 c.c. (= 0.001 c.c. immune serum).

5. To 1 c.c. of the fourth dilution (1 : 1000) add 9 c.c. of bouillon (= 1 : 10,000). Place 2 c.c. in a separate tube, and add 2 loopfuls of culture. Inject 1 c.c. (= 0.0001 c.c. immune serum).



FIG. 119.—REMOVING EXUDATE FROM THE PERITONEAL CAVITY OF A GUINEA-PIG (PFEIFFER BACTERIOLYTIC TEST).

A small incision is made through the skin, and the exudate is withdrawn by means of a pipet.

6. Control: Emulsify 2 loopfuls of culture in 2 c.c. of bouillon. Inject 1 c.c. This animal will probably succumb within twenty-four hours.

7. Control: To 2 c.c. of a 1 : 10 dilution of inactivated normal rabbit-serum add 2 loopfuls of culture and inject 1 c.c. intraperitoneally. If goats or horses are used in preparing the immune serum, this control should be conducted with normal goat- or horse-serum. According to Kolle, 1 loopful of virulent cholera culture is destroyed in the peritoneal cavity of a guinea-pig by 0.1 to 0.3 c.c. of normal rabbit's serum; 0.02 to 0.03 c.c. of normal goat's serum; 0.005 to 0.01 c.c. of normal horse's serum.

8. Control: A pig may be injected with 1 c.c. of a 1 : 100 dilution of the immune serum and with a loopful of some other micro-organism, such as *Bacillus coli*. This control, however, is not absolutely necessary.

An area of the abdominal wall of the guinea-pig about 1 inch in diameter

is shaved and cleansed with alcohol. After the injections have been made the bacteriolytic phenomena are observed.

In making this test fine capillary pipets are prepared and used for withdrawing the peritoneal exudate.

After permitting the animal to inhale a few drops of ether, to make sure that it will not suffer, a small incision is made through the skin of the abdomen. The capillary pipet, the large end of which is kept closed with the index-finger, is then quickly passed into the abdominal cavity. The index-finger is released, and the tube is gently moved about and withdrawn. As the result of capillary attraction sufficient exudate usually passes into the tube without the aid of suction (Fig. 119). The tube may be fitted with a rubber teat in case suction should be necessary. Hanging-drop and smear preparations are made and studied microscopically (Fig. 120).

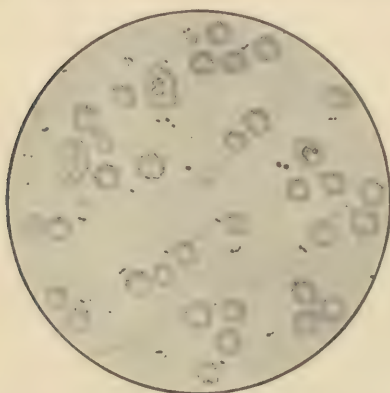


FIG. 120.—CULTURE OF CHOLERA UNDERGOING BACTERIOLYSIS. A POSITIVE PFEIFFER REACTION.

A hanging drop of peritoneal exudate removed from a guinea-pig one-half hour after injection with 1 c.c. of the suspension shown in Fig. 121 with 1 c.c. of 1:1000 dilution of cholera immune serum. Note that the bacilli are now quite short and coccoid in shape. At the end of seventy minutes the exudate was sterile. What appears to be two or three coccoid forms in apposition is really one bacillus undergoing lysis.

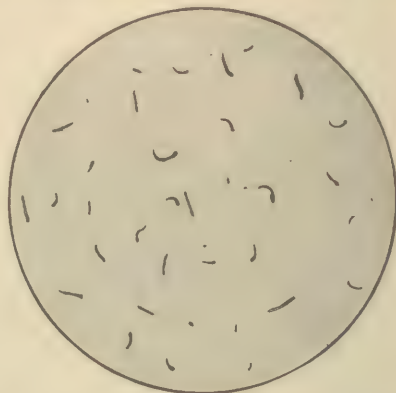


FIG. 121.—CULTURE OF CHOLERA BEFORE BACTERIOLYSIS. $\times 720$.

A hanging drop of cholera in normal salt solution prepared from a twenty-four-hour culture of cholera on agar-agar.

Stained smears are, however, less reliable and not so useful in determining the degree of bacteriolysis (Fig. 122).

It is best to withdraw the exudate immediately after injection, and then at intervals of ten, twenty, thirty, forty, and sixty minutes.

A hanging-drop preparation, made from the culture by emulsifying a minute quantity in bouillon, should be on hand as a control in studying bacteriolysis in the exudate (Fig. 121). A smear of the culture stained with dilute carbolfuchsin should also be on hand for making comparison with smears of the exudate (Fig. 123).

Bacteriolysis is first manifested by loss of motility. As the process progresses many of the bacilli become swollen and distorted, and later irregular and broken fragments or granules become apparent. Finally, at the end of an hour, the exudate is practically sterile. In those cases in which bacteriolysis is complete in an hour the animal generally survives. In the higher dilutions of serum bacteriolysis is incomplete, and the animal be-

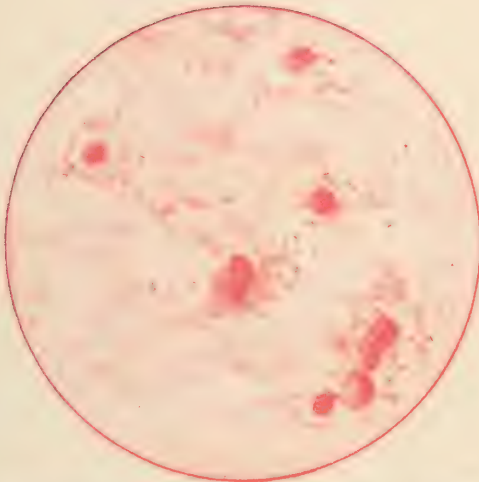


FIG. 122.—A SMEAR OF PERITONEAL EXUDATE REMOVED TWENTY MINUTES AFTER INJECTION OF CULTURE OF CHOLERA IN FIGS. 121 AND 123.
Stained with 1 : 10 carbolfuchsin.



FIG. 123.—STAINED PREPARATION OF CHOLERA BEFORE BACTERIOLYSIS.

comes toxic and may succumb after twenty-four hours, double the virulent dose being used in all injections.

In the foregoing titration a serum that is bacteriolytic in dilutions of 1 : 1000 or over will be satisfactory for purposes of diagnosis. When preserved in a sterile condition in separate ampules in a dark cold place the titer usually remains unaltered for several months.

Dried Serum.—If the serum is dried *in vacuo* it will be necessary to titrate with dilutions of the dried products in bouillon, as during the process of drying the amboceptor content may be decreased. Dried serum is to be preferred, however, as it keeps much longer and there is no danger of rendering it worthless by contamination. After drying, the product is ground in a mortar and stored in amounts of 0.1 or 0.2 gm. in separate ampules.

In determining the bacteriolytic titer of dried serum 0.1 gm. is carefully weighed out and dissolved in 9.9 c.c. of sterile bouillon. From this stock dilution other dilutions may be prepared in the manner previously described, and injected with a loopful of the culture.

After securing an immune serum of satisfactory potency the main test may be conducted. While the technic is more difficult than that of agglutination tests, the results, under proper conditions, are more conclusive, for there is less likelihood of group or partial amboceptor activity for closely allied bacteria taking place.

Technic of the Pfeiffer Test.—The suspected culture to be tested should be grown on agar for from eighteen to twenty-four hours, and used in doses of 1 loopful (2 mg.).

If cholera is suspected, cholera immune serum of known titer should be used. Dilutions of serum are made with sterile bouillon, and 2 c.c. of each dilution, representing two, five, and ten times the titer dose, are placed in separate glasses or small test-tubes. A fourth tube should contain 2 c.c. of a 1 : 100 dilution of normal serum, according to the animal used in producing the immune serum; a fifth tube should contain 2 c.c. of sterile bouillon (culture control).

Two loopfuls of suspected culture are thoroughly emulsified in each of the 5 tubes of the series, and 1 c.c. of each is injected intraperitoneally in 5 guinea-pigs weighing about 250 gm. each.

At intervals of ten, twenty, forty, and sixty minutes the peritoneal exudate should be removed with capillary pipets and examined in hanging-drop preparations. Smears may be prepared and stained with dilute carbol-fuchsin, although they give less information than hanging-drop preparations.

If guinea-pigs Nos. 1, 2, and 3 show granule formation at the latest after an hour, while in the fourth and fifth animals the bacteria remain whole, motile, and well preserved, the reaction may be regarded as positive and the diagnosis as established.

Pfeiffer Bacteriolytic Test *in Vivo* in the Diagnosis of Disease.—Bacteriolytins are usually produced somewhat later than agglutinins, and reach their highest point of production during convalescence. Bacteriolytic tests are used only for diagnostic purposes when agglutination reactions are negative or doubtful. The most satisfactory results from these tests are obtained in cholera. Bacteriolysis with typhoid bacilli is less typical and more incomplete; with the paratyphoid and dysentery bacilli it is even more unsatisfactory, and in anthrax, pest, and the various diseases due to cocci it does not occur.

The test is conducted in a manner similar to the preceding test, except

that instead of an immune serum the patient's serum is used, with a known culture of typhoid, cholera, paratyphoid, etc., according to the infection suspected to be present.

One c.c. of the patient's serum is secured in a sterile manner, inactivated by heating to 55° C. for one-half hour, and dilutions of 1 : 20, 1 : 100, 1 : 250, and 1 : 500 prepared with sterile bouillon. Two c.c. of these dilutions are placed in separate tubes, and 2 loopfuls of an eighteen-hour culture of the test organism emulsified in each. A fifth tube contains 2 c.c. of bouillon with 2 loopfuls of culture, and serves as the culture control.

One c.c. of each dilution and of the culture control is injected intraperitoneally into 5 guinea-pigs, and the exudate examined after twenty, forty, and sixty minutes.

If granule formation occurs in the first two or three animals, but is absent in the culture control, the reaction may be regarded as positive, and the diagnosis of typhoid, cholera, etc., considered as established.

If granule formation occurs to any extent in the fifth animal (culture control), the culture is to be regarded as unsuitable and the test repeated.

Measuring the Bactericidal Power of the Blood *in Vitro* by the Plate Culture Method (Stern and Körte).—Since the earliest days of bacteriology the aim and purpose have been to devise some means by which the bactericidal power of the blood could be measured, just as is done in testing an ordinary germicidal substance, namely, by adding a bacterial suspension to the serum and observing the effect on the bacterial content. This is shown by counting the bacteria in a loopful immediately after adding the bacterial suspension, and repeating this at regular intervals, the counting being done by the method of plate culture.

The use of the platinum loop in these tests is objectionable, since the dose is quite variable, depending upon whether the loopful is removed from the fluid edgewise, or with the loop held flat, like a spoon in use. If the serum contains agglutinins the results with any form of technic are likely to be irregular, and the number of colonies upon the plate stand in no relation to the actual number of micro-organisms present. The results may be masked by a rapid multiplication of the survivors, and accordingly several controls are necessary with any technic.

Neisser and Wechsberg have recommended the so-called *bactericidal plate culture method*. By this method the patient's serum is inactivated, and varying amounts mixed with definite and constant quantities of bacteria. To this a constant quantity of active normal serum is added as complement, to reactivate the bacteriolytic amboceptors. These mixtures are then incubated for several hours. To determine whether and in what proportion death of bacteria has resulted from the effect of the bacteriolysins, agar is added, and the mixtures are then plated and incubated for twenty-four hours or longer. The number of colonies are then counted and the results compared with control plates of the culture alone.

Stern and Körte have modified this technic slightly, and recommend the procedure as a substitute for the Pfeiffer test in the clinical diagnosis of typhoid fever. The method spares a certain number of animals, but it is somewhat more complicated than the Pfeiffer reaction, and its results are less trustworthy. As a clinical test, therefore, it is to be recommended only in cases in which the agglutination reactions have yielded uncertain results, although with practice and care the test oftentimes yields uniform and satisfactory results, and may be employed in making special investigations for determining the bactericidal power of the blood, as after typhoid immunization.

Technic of the Test.—The requisites for success are that all vessels and diluting fluids as well as the serums employed should be absolutely sterile. To secure uniform and reliable results it is necessary to familiarize oneself with the technic by repeated practice. In order to carry out the steps in the technic according to strict aseptic bacteriologic principles the services of an assistant are required.

Sterile bouillon is largely used throughout to maintain proper osmotic conditions.

With so many tubes and controls it is important that all tubes and Petri dishes be properly labeled with a wax-pencil to avoid confusion.

One c.c. of sterile patient's serum and an equal amount from a normal and healthy person to serve as a control are inactivated by heating to 55° or 56° C. for half an hour. These serums are then diluted 1 : 50 by adding 49 c.c. of sterile salt solution to each and mixing thoroughly.

Complement is prepared by securing 4 to 5 c.c. of sterile rabbit's blood and separating the serum. This serum is chosen because it should be from the same species of animal as that used in producing the immune serum to be tested. In determining the bactericidal titer of human serum either guinea-pig or rabbit complement serum may be used. Dilute 2 c.c. of this *fresh serum* (not over eighteen hours old) with 18 c.c. of *sterile* normal salt solution (1 : 10). Dose, 0.5 c.c.

Secure a good twenty-four-hour bouillon culture of typhoid bacilli (grown in the incubator) and dilute 1 : 500 by *thoroughly mixing* 0.1 c.c. of the culture in a flask containing 50 c.c. of sterile normal salt solution. This dilution of culture, when used in constant doses of 0.5 c.c., generally yields satisfactory results, but it may be necessary to try it out beforehand, for the control plates must regularly show a uniformly good growth and contain many thousands of colonies before uniform results can be expected. The bactericidal effect will then be distinctly shown by the reduction, in the proper plates, of this large number of colonies to zero or near it.

Place 1 c.c. of sterile neutral bouillon in each of 12 test-tubes which have been plugged with cotton, sterilized, and large enough to hold at least from 12 to 15 c.c. Place in the first of these tubes 1 c.c. of the diluted patient's serum and mix thoroughly by alternate sucking up and forcing out of the fluid; then, with the same pipet, draw up 1 c.c. and transfer it to the second tube of the series; mix as before, and transfer 1 c.c. to the third tube; continue in this manner to the last tube, from which, finally, 1 c.c. is discarded.

Each tube now contains 1 c.c. of fluid, representing dilutions of 1 : 100, 1 : 200, 1 : 400, 1 : 800, 1 : 1600, 1 : 3200, and so on up to 1 : 204,800 of the patient's serum. Higher or lower dilutions than these may be employed. It is very desirable that the dilutions be high enough to secure the limit of bactericidal activity, so that the last plates will show an increase in the number of colonies.

Arrange four tubes with the normal control serum, each containing 1 c.c. of fluid and representing the first four dilutions, viz., 1 : 100, 1 : 200, 1 : 400, and 1 : 800.

Label each tube carefully with the initials of the patient and the dilution it contains. Add 0.5 c.c. of the diluted bacterial emulsion, and finally 0.5 c.c. of the diluted complement serum to each tube.

All manipulations should be made with strict bacteriologic care and with the aid of an assistant, as the introduction of contaminating micro-organisms that may cause spore formation will considerably vitiate the value of the test.

The following controls are necessary:

1. 0.5 c.c. culture directly in a sterile Petri dish + 5 to 10 c.c. of neutral agar cooled to 42° C. and thoroughly mixed. This control will show the original number of bacteria contained in the emulsion. Mark as control No. 1.
2. 0.5 c.c. culture + 1.5 c.c. bouillon. Mark as control No. 2. After three hours' incubation this tube receives the usual amount of agar and is plated. It will show to what degree the culture has multiplied in the incubator.
3. Since the complement serum frequently contains bacteriolysin, it is necessary to control this element carefully. To a series of four tubes add the following doses of the serum, diluted 1 : 10: 1 c.c., 0.5 c.c., 0.2 c.c., 0.1 c.c. Add 0.5 c.c. culture to each tube, and sufficient bouillon to make the total volume in each tube 2 c.c.
4. 0.5 c.c. complement + 1.5 c.c. bouillon. To control the sterility of the complement serum.
5. 1 c.c. of patient's serum (1 : 100) + 0 c.c. bouillon. To control the sterility of this serum.
6. 1 c.c. of the control serum (1 : 100) + 1 c.c. of salt solution. To control the sterility of this serum.
7. 1 c.c. of the patient's serum in dilution of 1 : 100 + 0.5 c.c. culture + 0.5 c.c. bouillon. A control on the possible presence of complement in this serum.
8. 1 c.c. of the control serum in dilution of 1 : 100 + 0.5 c.c. culture + 0.5 c.c. bouillon. A control on the possible presence of complement.

All tubes with the exception of the first control which has been plated are placed in an incubator at 37° C. for three hours. At the end of this time the contents of each tube are plated in neutral agar. Sterile Petri dishes should be properly and plainly labeled with a wax-pencil and arranged in order. A flask of plain neutral agar is melted in boiling water and cooled to 42° C. The tubes are removed from the incubator and shaken gently, and with the aid of an assistant from 5 to 8 c.c. of agar are added carefully to each tube with a sterile 10 c.c. pipet. The contents are mixed by gentle rotation of the tube and then poured in the corresponding Petri dish, followed by an additional mixing according to the usual bacteriologic procedure. With ordinary speed the whole set of tubes may be poured in a satisfactory manner before the flask of agar has had time to harden.

Another method of plating consists in pipeting the contents of each tube into its corresponding dish, and then washing the tube with an additional 1 c.c. of sterile salt solution to remove all traces of serum and culture. Or the end of each tube may be flamed and the contents poured directly into a dish. If this method is adopted small test-tubes should be used. While the method is more convenient, it is usually not so accurate as the first two methods.

Neisser plates but 5 or 10 drops from each tube. The dose decided upon is the one employed throughout. For example, it would be erroneous to take 5 drops from one tube and 10 from another. Neisser, however, uses much smaller amounts of the serum, as 1, 0.3, 0.1, 0.03, and 0.01 c.c., instead of the much higher dilutions given in this technic. These differences must be remembered and the proper dilutions employed, and but 5 to 10 drops should be plated in performing the bactericidal plate test according to Neisser's technic.

Töpfer and Jaffe pour a thin layer of agar into a Petri dish and allow it to harden. Upon this they pour the culture-serum agar mixture, which

after settling, is covered with another thin layer of agar. In this way a culture in the water of condensation is avoided. In the usual technic this may be avoided by turning the plates over soon after hardening occurs so that the water of condensation collects on the cover.

All plates are incubated at 37° C. for from twenty-four to thirty-six hours and the colonies then counted. In some plates the number may be so large that counting will be inaccurate and unnecessary. Significance can be attached only to marked and easily recognizable differences. According to Neisser, the growth is best and most rapidly described by means of approximate estimates, using a scheme somewhat like the following: 0 or almost none; about 100; several hundreds; thousands; very many thousands; infinite numbers. A distinct bactericidal action is then present only if the controls react normally, and if a reduction of colonies from an infinite number or many thousands to 0 or very few has occurred. As previously stated, the test can then be regarded as satisfactory only if the lower limits of bactericidal activity have been reached and the last plates show an increase in the number of colonies. Examination of these plates is very much facilitated by using a colony counter, that of Stewart being particularly serviceable with agar plates.

The controls are first examined and the results recorded, and finally those of the patient's serum are set down.

As a practical illustration, the results of a test with a rabbit typhoid immune serum are given, because this is in general an average example of those usually obtained:

1 C.C. DILUTIONS OF IMMUNE SERUM.	TWENTY-FOUR- HOUR CULTURE OF BACILLUS TYPHOSUS (DILU- TION 1 : 500 C.C.).	RABBIT COMPLE- MENT SERUM, 1 : 10 C.C.	PLATES POURED AFTER THREE HOURS AT 37° C. COUNTED AFTER TWENTY-FOUR HOURS	
			Immune Serum (Inactivated)	Normal Rabbit Serum (In- activated).
1 : 100.....	0.5	0.5	About 500 colo- nies.	Many thousand.
1 : 200.....	0.5	0.5	About 125 colo- nies.	Many thousand.
1 : 400.....	0.5	0.5	Sterile.	Many thousand.
1 : 800.....	0.5	0.5	Sterile.	Many thousand.
1 : 1600.....	0.5	0.5	Sterile.	
1 : 3200.....	0.5	0.5	Two colonies; practically sterile.	
1 : 6400.....	0.5	0.5	100 colonies.	
1 : 12,800.....	0.5	0.5	About 800 colo- nies.	
1 : 25,600.....	0.5	0.5	About 2000 colo- nies.	
1 : 51,200.....	0.5	0.5	Many thousand.	
1 : 102,400.....	0.5	0.5	Many thousand.	
1 : 204,800.....	0.5	0.5	Many thousand.	

CONTROLS

Control 1: 0.5 c.c. culture plated immediately: many thousands of colonies.

Control 2: 0.5 c.c. culture plated after being incubated three hours: plate very crowded; number of bacteria increased.

Control 3: Varying amounts of normal rabbit serum used as complement.

The first plate containing 0.1 c.c. serum showed a slight bactericidal action; the remaining plates showed many thousand of colonies and were comparable to control No. 1.

Control 4: 0.5 c.c. complement serum: sterile.

Control 5: 0.01 c.c. inactivated immune serum: sterile.

Control 6: 0.01 c.c. inactivated normal control serum: plate shows several colonies of contaminating bacteria.

Control 7: 0.01 c.c. inactivated immune serum plus culture: plate shows many thousands of colonies.

Control 8: 0.01 c.c. of inactivated normal serum plus culture: many thousands of colonies.

An examination of this experiment shows that the dose of culture was satisfactory, that the culture increased during the three hours of primary incubation, that the complement serum was very slightly bactericidal in a dose lower than that used in making the test, and that the technic was fairly satisfactory, slight contamination being present in but one plate—that of the inactivated normal control serum.

The most striking result observed is the absence of complete bactericidal activity in the first two plates, which contained the largest amount of immune serum, and where one would naturally expect to find complete sterility. The titer of this serum was between 1 : 3200 and 1 : 6400. According to Neisser and Wechsberg these paradoxical results are caused by deviation or "deflection of complement," as was explained in a previous chapter. In bactericidal experiments according to Neisser, the deflection is caused by an excess of amboceptors in the immune serum. In a mixture of bacteria, complements, and large amounts of amboceptor the complement is bound not only by the amboceptors anchored to the bacteria but also in large measure by "free" amboceptors that are not anchored to bacteria. A portion of the anchored amboceptor, therefore, finds no complement at its disposal, and is, therefore, unable to exert any bactericidal action, which gives rise to a relative lack of complement.

This phenomenon resembles the action of agglutinoids in the agglutination reaction, where, in the lowest dilutions, agglutination is feeble or absent, but becomes manifest in the higher dilutions.

In the foregoing experiment the immune serum used was several weeks old; perfectly fresh serums are not so likely to show this so-called "deflection of complement." In many instances, and especially if a fresh serum is used, one cannot help thinking that agglutinins may be responsible for the absence or diminution of bactericidal activity in the lower dilutions. It is certainly true that hemagglutinins considerably inhibit hemolysis, and this is especially the case with serums of low hemolytic activity. With more potent serums the agglutinins are diluted until activity ceases and hemolysis is ready and complete. Reasoning from analogy, therefore, the absence or diminution of bactericidal activity may be due to agglutinins, and the theory of "deflection of complements" may be emphasized a little too strongly.

According to Halm, normal human serums show bactericidal activity in only about one-third of the cases, and the titer is only very exceptionally demonstrable in dilutions higher than 1 : 500. The serums of advanced cases of typhoid fever or of those but recently recovered are bactericidal in dilutions greater than 1 : 1000, and may reach 1 : 50,000 or higher. Similarly after typhoid immunization the patient's serum may show a high

bactericidal titer. Weston has found such serums active in dilutions of 1 : 20,000, higher dilutions not being used.

Besides being used in typhoid, the plate culture method has been employed for experimental purposes in cholera, dysentery, paratyphoid, and other infections with bacilli of the typhoid-colon group. With these, however, the test possesses but little diagnostic significance.

The bactericidal titer does not run strictly parallel with the agglutinins or complement-fixing bodies.

Measuring the Bactericidal Power of the Blood by Capillary Pipet Method (after Wright¹).—By this technic it is sought to overcome the fallacies of the "loopful" method of measurement and those due to agglutination of the test organism.

The native complements of the patient's own serum are used; hence the serums used in this technic must be fresh. Quantitative titration is accomplished by furnishing varying dilutions of culture, with a constant quantity of serum. A series of volumes of serum is taken, and to these are added equal quantities of progressively increasing dilutions of a counted bacterial culture. The mixtures are kept at 37° C. for twenty-four hours, after which each is introduced into nutrient broth and cultivated to see whether a complete bactericidal effect has been exerted. *The largest number of bacteria that a constant quantity of serum has been able to kill furnishes a measure as to its bactericidal power.*

After a few technical details have been mastered this method is quickly performed and yields fairly constant and reliable results. It is not adapted for the titration of old immune serums, but is a ready clinical test, finding a special field of usefulness in determining the bactericidal powers of the blood after typhoid immunization.

Requisites for Carrying Out the Test.—1. A specimen of the patient's blood is collected aseptically, a process that may be accomplished by thoroughly cleansing the finger with alcohol and collecting blood in a sterile Wright capsule or, better perhaps, by means of venipuncture, when from 2 to 5 c.c. may be collected aseptically in a sterile centrifuge tube. The control blood from a healthy individual may be collected in a capsule. The serums are carefully separated and pipeted into small sterile test-tubes; 1 c.c. of each is ample for the test.

2. A twenty-four-hour-old broth culture of the test organism (a young culture is required, because such a culture contains a few dead micro-organisms and the absorption of bactericidal elements by dead organisms is thus avoided).

3. About two dozen "looped pipets" made according to the directions given in Chapter I.

4. Sterile neutral broth for making dilutions and cultivations. This is prepared and sterilized in the usual manner, from 5 to 10 c.c. being placed in test-tubes. When working with the typhoid bacillus a special broth containing 1 per cent. of mannite and sufficient litmus to color it a deep blue (Smallman) should be on hand.

5. Two dozen small test-tubes, plugged and sterilized, for making dilutions of the culture.

Preparation and Enumeration of the Bacterial Culture.—As the principle of the test depends upon measuring the bactericidal activity of the blood according to the number of organisms that are killed, it is necessary to prepare somewhat high dilutions and count the number of organisms in a unit volume in each dilution.

¹Wright, A. E.: *Technique of the Teat and Capillary Glass Tube*, 1912, London, Constable & Co.

For this purpose place 10 sterile test-tubes in a rack, and arrange 10 sterile Petri dishes on the table to correspond to these. To the first tube add 4.9 c.c. of plain sterile broth; to the second, fourth, sixth, eighth, and tenth tubes add 1 c.c., to the third, fifth, seventh, and ninth tubes add 4 c.c.

With a sterile graduated 1 c.c. pipet add 0.1 c.c. culture to the first tube and then discard the pipet by placing it in a cylinder of disinfecting solution or hot water. With a second sterile pipet mix the contents of tube No. 1 by carefully sucking it in and forcing it out of the pipet several times, and place 0.1 c.c. in Petri dish No. 1. Then with the same pipet transfer 1 c.c. to the second tube, mix well, and place 0.1 c.c. in Petri dish No. 2; next transfer 1 c.c. to the third tube, mix, and place 0.1 c.c. in the third Petri dish. Continue in this way until the last tube is reached, when 1 c.c. is discarded into a germicidal solution and 0.1 c.c. placed in the tenth Petri dish. To each Petri dish now add from 8 to 10 c.c. of neutral agar at 41° C., and mix thoroughly. After the plates have hardened they are turned over in order that the water of condensation may collect on the cover. They are then incubated for twenty-four hours, and the colonies carefully counted.

We now have the following dilutions of culture: 1 : 50, 1 : 100, 1 : 500, 1 : 1000, 1 : 5000, 1 : 10,000, 1 : 50,000, 1 : 100,000, 1 : 500,000, and 1 : 1,000,000. Since but 0.1 c.c. of these dilutions were plated, the total number of colonies in each plate must be multiplied by 10 in order to obtain the approximate number per cubic centimeter of the various dilutions.

To secure fairly uniform results the various dilutions must be thoroughly mixed and the pipeting be accurately performed. We have found that this method usually gives better results than are obtained by plating but one or two of the higher dilutions, which are used as a basis for calculating the number of bacteria in the other dilutions.

Filling the Pipets.—With a wax-pencil make a mark upon the capillary stem of a sterile looped pipet at a point 2 cm. from the lower end, and fit a rubber teat to the barrel. The point of the capillary stem is now broken off between the finger and thumb, the lower portion is sterilized in the flame, and the air is expelled from the teat.

Mannite broth is then aspirated into the pipet until the bulb is about two-thirds full and the capillary portion contains an air column rising to at least 5 cm. from the end (Fig. 124).

The end of the capillary stem is now inserted into the tube containing the patient's serum, and the serum is allowed to flow in until it reaches the pencil mark.

The orifice of the pipet is now raised above the surface of the serum, and a small bubble of air is admitted into the tube to serve as an index for the measurement. The end of the capillary stem is now carried into the tube containing the highest dilution of the organism, and the culture is allowed to flow in until the bubble of air has been carried just past the pencil mark.

The serum and culture must now be mixed, being careful not to contaminate the broth. This is effected by blowing these two volumes out into a sterile mixing tube and drawing up and blowing out the fluid several times in succession. By starting with the highest dilution the same mixing tube may be used throughout the series. With an air-bubble of at least 5 cm. between serum and broth this can be quite easily achieved without driving the sterile broth down from the bulb of the pipet into the lower part of the capillary stem and contaminating it there.

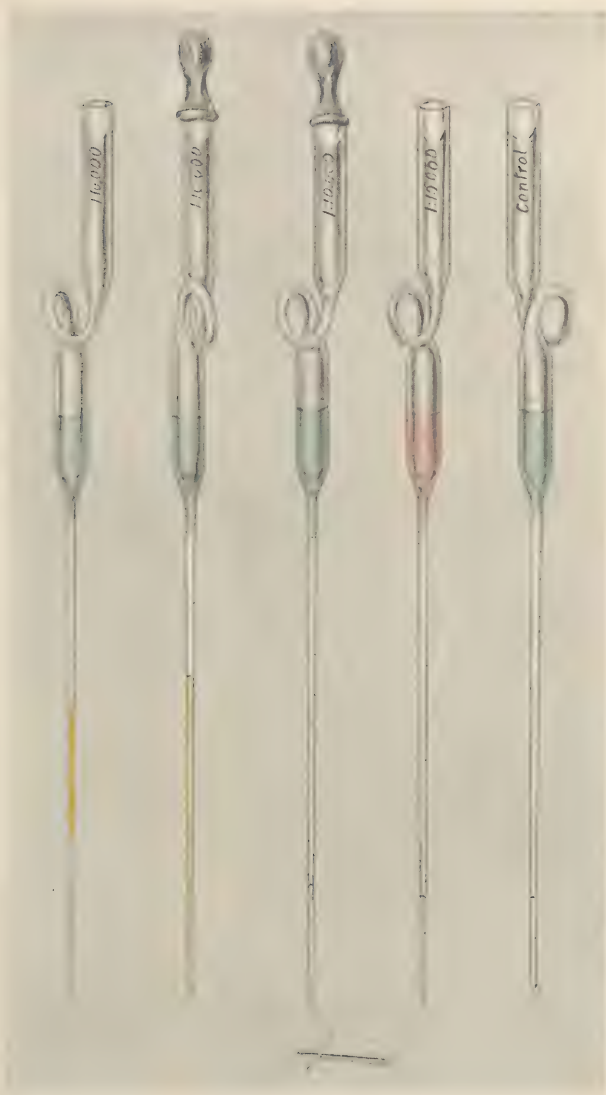


FIG. 124.—BACTERICIDAL TEST (LOOPEO PIPET METHOD OF WRIGHT).

The pipet shown on the extreme left contains the mannite-litmus broth and an equal volume of patient's serum and emulsion of typhoid bacilli; the second pipet shows the serum and bacillary emulsion mixed; the middle pipet shows the serum-bacillary mixture cultured in the broth; the fourth pipet shows acid production in the broth by living bacilli which escaped destruction after twenty-four hours' incubation; the fifth pipet (extreme right) is the serum control. The broth, being clear and unchanged, shows that the serum was sterile.

The column of mixed serum and culture is now drawn up into the middle region of the capillary stem and the lower end of the tube is sealed. The teat is then removed, and the dilution that has been used is written on the barrel of the pipet.

The series of pipets are now filled with the nine measuring dilutions of the culture.

Controls.—The serum from a healthy individual or, better still, the pooled serums of several persons may be used in precisely the same way, with at least the second, fourth, sixth, and eighth dilutions of culture.

Several culture controls are advisable. The pipets are filled with mannite broth in the usual manner, and then with one volume of at least four different dilutions, usually 1 : 50, 1 : 5000, 1 : 100,000, and 1 : 1,000,000. The tubes are sealed, labeled, and incubated together with those concerned in the test proper.

One pipet is to contain the usual quantity of broth and one volume of the patient's serum. This is a control on the sterility of the patient's serum. A similar preparation is made with the control serum to serve as a control on its sterility.

When the whole series of tubes have been filled, these are placed upright in a large test-tube or cylinder labeled with the date and the source of the serum, and incubated at 37° C. for from eighteen to twenty-four hours.

Test for the Germicidal Activity of the Serum.—The serum and culture in the capillary portion of the tube must now be mixed with the broth in the barrel. This is accomplished by taking each pipet in hand singly, and heating the lower portion of the capillary stem in a "peep-flame" and drawing out into a small thread with a pair of forceps.

A collapsed teat is now fitted over the barrel, and the negative pressure carefully regulated by keeping the finger and thumb in position on the teat, and the finely drawn out end of the capillary stem gently snapped across. The column of serum and culture will then be carried up into the bulb of the pipet. The end of the tube is now sealed.

When the whole series of pipets has been dealt with in similar fashion they are returned to the incubator for another twenty-four hours.

Reading the Result.—The continued sterility of the broth may be determined from direct naked-eye inspection.

When a complete bactericidal effect has been secured, the broth will have undergone no color change, but will still be clear.

When a growth of the typhoid bacillus has occurred, a perceptible cloudiness will be apparent in the broth, and the color will have changed from blue to reddish blue, indicating that acid has been formed from the mannite.

Turbidity of the broth without a change of color would indicate the admission of contaminating microbes that were unable to split mannite with the formation of acid.

The controls are first examined and recorded. All the culture controls should show a growth and change of color. The serum controls should be sterile; the normal serum controls may or may not be sterile, depending upon the amount of natural bactericidal amboceptors which it contains for the test organism.

The pipets containing the patient's serum are now examined, and a simple numeric expression for the result is obtained by referring to the result of the enumeration of the various dilutions, as determined by the counting of the plates. In this manner the number of bacteria contained in 1 c.c. of the lowest dilution of the bacterial culture that has been com-

pletely sterilized is calculated. This will give the number of micro-organisms which 1 c.c. of serum would be capable of killing.

Although this test affords a convenient basis for the comparison of serums, it must be understood that the expression is entirely arbitrary, and will vary according to the culture employed; this is true of any technic for determining the bactericidal titer of a serum.

The Lacy-Heist Method of Determining the Bactericidal Activity of Coagulable Blood.—Heist, Cohen and Cohen¹ have described this technic; it is simple and usually yields decisive results. These investigators have shown by means of this test that the pneumococcus is able to grow in the coagulable blood of susceptible animals, as the mouse, whereas, in the blood of insusceptible animals, as the pigeon, growth does not occur or is feeble. Kolmer and Matsunami² have successfully employed the method in a study of natural immunity to the meningococcus. Increasing the virulence of pneumococci for rabbits was found to increase their ability to grow in rabbit blood *in vitro* according to the results observed with this method; the test possesses an approximate quantitative value, and is regarded by Heist and Cohen³ as a better measure of pneumococcus immunity than the agglutination test. By means of this method it has been shown that whole blood before it coagulates is much more pneumococcidal than the serum, and immunization with pneumococcus bacterins has been found to increase this hemocidal activity.

The technic is as follows:

1. Heavy walled glass tubing is drawn out into capillary tubing having an inside diameter of 0.5 to 1 mm. The capillary tubing is cut in 15 cm. lengths and the ends trimmed square by nicking with a fine file or with the edge of a carborundum pocket hone, and breaking across. Five of these tubes are held side by side, palisade fashion, and a pellet of plasticine molded closely about them near one end. A short piece of glass tubing stuck into the plasticine so that it encloses the five protruding ends makes a convenient handle. Pressure on the plasticine spreads out the capillary tubes into a fan shape. With a wax-pencil a fiduciary mark is made upon each capillary tube about 5 cm. from its free end and also each one is given an identification mark. Tube 1 receives one dot with the wax-pencil, tube 2, two dots, and so on. This many stemmed pipet is a modification of that devised by Wright for estimating the coagulation time of blood (Fig. 125).

2. Five small, sterile test-tubes are numbered from 1 to 5 and arranged in a row. They may be very conveniently held in a sloping position by thrusting their ends in a long strip of plasticine. In tube 1 is placed a quantity of a twenty-four-hour culture of pneumococci in blood broth. In tube 2 one volume of the broth culture is diluted with four volumes of saline solution, making a 1 : 5 dilution. In a similar manner a 1 : 25 dilution is made in tube 3, and a 1 : 125 and a 1 : 625 in tubes 4 and 5. In other words, dilutions of culture are made in multiples of five.

3. The many stemmed pipet is taken up and the tip of tube 1 touched to the surface of the undiluted culture in test-tube 1. The fluid runs up the slender tube by capillary attraction. When the upper level of the ascending fluid reaches the fiduciary mark, the tip of the tube is withdrawn from the test-tube, the fluid which has run up the tube remaining *in situ*. The other capillary tubes are filled in the same manner from the test-tubes bearing corresponding numbers. If now the tip of one of the capillary tubes filled with diluted culture is touched to a piece of moist cheese-cloth the column of fluid will run out, the adhesion between the cloth and the

¹ Jour. Immunology, 1918, 3, 261. ² Ibid., 1918, 3, 201. ³ Ibid., 1919, 4, 147.

liquid used being greater than that between the same liquid and glass. If the tube should not completely empty itself, gentle blowing into the other end of the pipet will start the downward flow again. In this manner each capillary tube is thoroughly emptied in turn.

4. The ear of a rabbit is rubbed briskly for a moment to increase the circulation and a vein is pricked. As the blood wells up the tip of capillary tube 1 is introduced into the drop and the blood flows up by capillary attraction. When the ascending column of blood has reached the fiduciary mark the tube is moved aside and tube 2 takes its place in the flowing blood. In less than a minute all five tubes are filled.

5. When all five tubes have been filled the distal ends are sealed by dipping them in paraffin which has been melted and allowed to cool until it is on the point of solidifying. This method of closing the ends avoids bringing any appreciable amount of heat in contact with the freshly coagulated blood. The capillary tubes are then gently pulled out of the pellet of plasticine, dropped into a test-tube and incubated for twenty-four hours, at the end of which time they are taken out, the tips broken off, and a drop from each blown out upon a glass slide. All five drops can be placed in a row on the same slide. The dots which have been placed upon the capillary tubes before beginning operations enables one to identify them. The slide is fixed in formalin vapor, stained, and examined under the microscope.

6. Under the microscope it is seen that some tubes contain no pneumococci. Numerous red and white blood-corpuscles are present and well stained, and strands of fibrin are interlaced among the cells. Other tubes contain great numbers of pneumococci mixed with debris, but very few cells; apparently the cells and fibrin have been digested by the pneumococci. These two pictures are practically the only ones encountered. Either the pneumococci have grown vigorously or they have not grown at all.

Matsunami¹ has applied this test to a study of the meningococcal activity of normal and immune sera and found that it could not be accepted for the purpose of measuring the degree of meningococcal activity of an immune serum, but that the method was useful for measuring the degree of natural immunity. Very probably the test has its limits for measuring the degree of bactericidal activity of immune serum or blood because it utilizes the native complement contained in the volume of blood being tested, which may be insufficient for complementing all of the bacteriolytic amboceptors present.

More recently Borow and the writer² have found the test of value in the course of chemotherapeutic studies in bacterial infections, medicaments being injected into rabbits by various routes and in doses per body weight, the tests being conducted before and at intervals after the administration for the purpose of determining if bactericidal activity of the blood was increased, and if so, how long this was maintained.

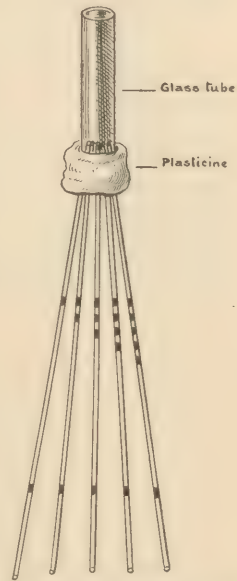


FIG. 125.—WRIGHT'S MANY STEMMED PIPET.

Used in estimating the bactericidal activity of whole coagulable blood according to the method of Heist and Lacy.

¹ Jour. Immunology, 1920, 5, 51.

² Jour. Infect. Dis., 1922, 31, 116.

BACTERIOLYTIC SERUMS IN THE TREATMENT OF DISEASE

While bacteriolysis is readily demonstrated with some bacteria both *in vivo* and *in vitro*, nevertheless, when such serums are used therapeutically, beneficial results are not dependent solely upon lysis of the infecting bacterium, but are usually the result of a combination of lysis with increased phagocytosis, due to the simultaneous presence of bacteriotropins (immune opsonins). When one recalls the close similarity in general properties that exists between bacteriolysins and bacteriotropins, the difference between extracellular and intracellular lysis is relatively slight, and tends to strengthen Metchnikoff's views regarding the close relationship of the bacteriolysins to leukocytic products and phagocytosis. While extracellular lysis can occur in the absence of cells, especially *in vitro*, yet in the administration of antibacterial serums the activities of the leukocytes are much in evidence, extracellular lysis or bacteriolysis proper being rather subsidiary to intracellular lysis or phagocytosis.

The preparation and methods of standardization of antibacterial serums are given in the chapter on Passive Immunization. Most effort in this direction has been expended on the preparation of antiserums for the treatment of meningococcic, streptococcic, pneumococcic, and gonococcic infections, and the greatest success has been attained with the various anti-meningococcic serums.

CHAPTER XX

HEMOLYSINS

Hemolysis is the term applied to the solution or lysis of red blood-corpuscles. Strictly speaking, it would include the disintegration and solution of the stroma, although in practice the term is applied to any process in which the cells are so injured as to liberate hemoglobin into the surrounding fluids, with or without solution of the stroma.

KINDS OF HEMOLYTIC AGENTS

Hemolysis or erythrocytolysis may be caused by various *non-specific* physical and chemical substances which act alike upon the erythrocytes of a large group of animals, and *specific* agencies which act upon the corpuscles of one kind of animal. For example, the bacterial hemolysins are non-specific and will hemolyze human corpuscles and those of the lower animals; serum hemolysins, however, are highly specific, and that for human corpuscles will act only upon these cells. These hemolytic substances may be grouped as follows:

- | | | |
|--------------|---|--|
| Non-specific | { | 1. Mechanical agitation. |
| | | 2. Temperature changes (heat; alternate freezing and thawing). |
| | | 3. Water; hypotonic and hypertonic saline solutions. |
| | | 4. Acids, salts, and other chemical substances. |
| | | 5. Tissue extracts (autolytic products). |
| | | 6. Inorganic colloids (silicic acid). |
| | | 7. Photodynamic substances (dyes, etc.). |
| | | 8. Vegetable poisons (ricin; abrin, croton, curcin, and robin);
"saponin substances." |
| | | 9. Bacterial poisons (tetanolysin; streptolysin; staphylolysin, etc.). |
| | | 10. Animal secretions (spider poison; cobra venom, etc.). |
| Specific | { | 1. Natural serum hemolysins. |
| | | 2. Immune serum hemolysins. |

Hemolysis by Mechanical Agitation; Temperature Changes.—Red blood-corpuscles collected by whipping blood to prevent coagulation are readily ruptured; these corpuscles also appear to become slightly more susceptible to the hemolytic activity of hypotonic salt solution, serum hemolysins, and other specific and non-specific hemolytic agents. For this reason corpuscles are generally secured by collecting the blood in oxalate or citrate solutions when fragility tests are to be conducted.

Alternate freezing and thawing results in hemolysis. Heating to 62° to 64° C. causes hemolysis of the corpuscles of warm-blooded animals, the blood-cells of cold-blooded animals being slightly more susceptible. I have found that sheep corpuscles may be heated to 55° C. for fifteen minutes without undergoing hemolysis.¹

Hemolysis by Water, Hypotonic, and Hypertonic Saline Solutions.—As is well known, red blood-corpuscles undergo rapid hemolysis in water. The intravenous injection of large amounts of water may produce hemolysis

¹ Amer. Jour. Syph., 1921, 5, 628.

in vivo with the production of a febrile reaction, hemoglobinuria, and other symptoms.

Solutions for intravenous injection should be *isotonic*, and for ordinary purposes 0.7 to 1 per cent. solutions of sodium chlorid in water suffice. The average is 0.85 per cent. sodium chlorid in water, well known as *physiologic or normal saline solution*, and employed for the preparation of red corpuscle suspensions in complement-fixation and other hemolytic tests.

Hypotonic saline solutions contain less than 0.7 per cent. sodium chlorid and *hypertonic* solutions contain more than 1 per cent. The fresh red blood-corpuscles of healthy human beings do not begin to hemolyze in hypotonic saline solutions until the strength is reduced to approximately 0.45 per cent.; in hypertonic solutions hemolysis may be very slight at approximately 7 per cent., but is not marked or complete even at 10 per cent. The resistance of corpuscles varies considerably among different persons and lower animals in health and disease as discussed in the succeeding paragraph; preserved red blood-corpuscles and blood prepared by defibrination are more fragile than fresh corpuscles collected in citrate solution.

Hemolysis in water and hypotonic saline solutions appears to be essentially a physical process for establishing osmotic equilibrium. Water enters the corpuscles, injuring the stroma, and thereby permitting the escape of hemoglobin; before this occurs the suspension of cells is opaque because of the obstruction to light offered by the corpuscles, but on the completion of hemolysis ("laking") the solution is perfectly transparent and the stroma settles to the bottom.

Hemolysis by hypertonic saline solutions is apparently due to the action of sodium chlorid penetrating the stroma.

Variation in the Resistance of Red Blood-corpuscles to Hypotonic Saline Solutions.—Many investigators have studied the tonicity of red blood-corpuscles by placing the cells in varying solutions of sodium chlorid and other salts in water to determine the concentration where hemolysis begins (point of *minimal resistance*) and where it is complete (point of *maximal resistance*). With solutions of sodium chlorid the following *average* values have been reported for the red blood-corpuscles of normal healthy human beings:

	Beginning hemolysis (minimal resistance)	Maximal resistance (complete hemolysis).
Ribierre ¹	0.44	0.34
Chauffard and Rendu ²	0.46	0.38
Paoloni ³	0.44	0.36
Hill ⁴	0.457	0.34
Morris ⁵	0.47	0.3
Fontaine ⁶	0.45	0.27
Greenthal and O'Donnell ⁷	0.425	0.325

Smith and Brown⁸ found the red corpuscles of different healthy horses to vary in resistance to hypotonic saline solutions, and the same is true of the corpuscles of human beings. For this reason minor degrees of variation from the average are of little or no significance.

Variation in results may be influenced by the temperature at which the

¹ Thesis de Paris, 1903, 106.

² Presse méd., 1907, 15, 345.

³ Policlinico, 1913, No. 6, 243.

⁴ Archiv. Int. Med., 1915, 16, 809.

⁵ Clinical Laboratory Methods, Appleton & Company, 1913, 264.

⁶ Jour. Amer. Med. Assoc., 1921, 77, 1022.

⁷ Amer. Jour. Dis. Child., 1921, 22, 212.

⁸ Jour. Med. Research, 1906, 15, 425.

tests are made and the time elapsing before the reactions are read; also according to whether the corpuscles are used in citrated blood or after being washed. Serum appears to protect the corpuscles to a slight degree. In my own experience the corpuscles of healthy persons have yielded the following average results when tested by the method described in this chapter under Practical Applications:

Washed corpuscles: 0.44 (initial); 0.32 (complete).

Citrated blood: 0.45 (initial); 0.36 (complete).

Whole blood: 0.45 (initial); 0.38 (complete).

The point of initial or beginning hemolysis is more definite in my experience than the point of complete hemolysis and the more valuable of the two.

Many attempts have been made to place the tonicity test on a diagnostic basis, but with little success. In 1907 Chauffard showed that there was an increased fragility of the erythrocytes in chronic family (hemolytic) jaundice which has been confirmed by numerous investigators. In obstructive jaundice, however, resistance is generally increased, so that the fragility test possesses practical value in differentiating between hemolytic and obstructive jaundice.

In secondary anemias there are apparently no constant values for the points of beginning and complete hemolysis, although in many cases there is a slight decrease of resistance. In pernicious anemia the values are likewise inconstant, but generally the cells are more fragile than normal blood or in secondary anemias. Hill has found the following averages based upon a study of the blood of 19 normal persons, 24 with secondary anemia, and 13 with pernicious anemia:

Normal: 0.457 (initial); 0.340 (complete).

Secondary anemia: 0.475 (initial); 0.322 (complete).

Pernicious anemia: 0.477 (initial); 0.322 (complete).

Butler¹ has observed increased resistance in some cases of sepsis and acute infectious diseases; Greenthal and O'Donnell, however, were unable to observe constant changes in scarlet fever, diphtheria, and other infectious diseases, although in some cases of great severity a distinct increase in resistance was noted. Pel,² Karsner and Pearce,³ and Hill⁴ have observed an increased resistance after splenectomy, and especially the point of maximal resistance. The administration of arsenic (Fowler's solution, arsphenamin, etc.) has been observed to increase the resistance of erythrocytes to hypotonic saline solutions.

A method for conducting the tonicity test is described in this chapter under Practical Applications.

Hemolysis by Acids, Salts, and Other Chemical Substances.—Organic and inorganic acids and alkalis are extremely hemolytic for the corpuscles of all animals. The practical application is in relation to the preparation of glassware for complement-fixation and other hemolytic reactions. Brown and myself⁵ found that 1 c.c. of a 1 : 300 dilution of normal solution of hydrochloric acid, equivalent approximately to 0.000012 c.c. or about 0.000015 c.c. of commercial acid (81 per cent.), proved hemolytic when added to 1 c.c. of a 2½ per cent. suspension of washed sheep corpuscles. Approximately 1 c.c. of a 1 : 100 dilution of normal sodium hydroxid (about 0.0004 gm.) was likewise hemolytic.

Chemical agents produce hemolysis by changes in the stroma probably by dissolving the lipoids or hydrolyzing the proteins and destroying the

¹ Quart. Jour. Med., 1912, 6, 145.

² Deut. Arch. f. klin. Med., 1912, cvi, 592.

³ Jour. Exper. Med., 1912, 16, 769.

⁴ Arch. Int. Med., 1915, 16, 809.

⁵ Amer. Jour. Syph., 1919, 3, 8.

power for retaining hemoglobin. Therefore, only such chemical agents as may penetrate corpuscles are actively hemolytic, as ammonium salts, alcohols, ketones, esters, antipyrin, amids, neutral amino-acids, urea, bile acids and their salts, etc.; likewise lipid solvents, as chloroform, ether, alcohol, by dissolving lecithin and cholesterol of the stroma. Sodium bicarbonate solutions of 1 or 2 per cent. are hemolytic, but $\frac{1}{10}$ per cent. solutions are not hemolytic. Arseniuretted hydrogen, numerous anilin compounds, nitrobenzol, etc., are hemolytic when inhaled and bear an important relation to industrial medicine. Curiously, some of these substances, as AsH_3 , are hemolytic in the living body, but not in the test-tube, producing their effects through tissue changes, as shown by Friedberger and Brossa¹ and others.

Hemolysis by Tissue Extracts (Autolytic Products).—These are of practical importance in relation to immunologic reactions in connection with the preparation of tissue extracts employed as antigens for the Wassermann test. Saline extracts of tissues may contain specific hemolysins extracted from the blood contained in the organ or even from the cells themselves of such tissues as bone-marrow and spleen. Decomposition products are especially hemolytic, and particularly of the liver, due to the presence of fatty acids and bile-salts. Alcoholic extracts of fresh tissues, and particularly those of muscle, are but feebly hemolytic, whereas extracts of fatty tissues are highly hemolytic.

Hemolysis by Inorganic Colloids.—Landsteiner and Jagic² have observed the agglutinating and hemolyzing activities of silicic acid, and several investigators have studied its hemolytic activity and especially in combination with lecithin and serum. So far all attempts toward utilizing mixtures of silicic acid and serum as hemolysin for complement-fixation tests have not met with success.³

Hemolysis by Photodynamic Substances.—Sacharoff and Sachs,⁴ Pfeiffer,⁵ Hausmann,⁶ and others, have observed the hemolytic effects of various dyes, chlorophyll, hematoporphyrin, urobilin, and other substances, but the subject does not appear to possess sufficient importance in relation to immunology to warrant further discussion.

Hemolysis by Vegetable Poisons.—A number of plant poisons, the so-called "vegetable toxalbumins," have been discussed in preceding chapters in connection with the toxins and antitoxins as owing a portion of their toxic effects to hemolytic activity. Of these, crotin and curcin are especially hemolytic, while ricin, abrin, and crotin are feebly hemolytic, but actively agglutinating. While their effects are non-specific, yet they vary greatly according to the species of animals whose blood is used.

The "saponin substances," classed as glucosids and found in at least forty-six different families of plants, are active hemolytic poisons. The mechanism of hemolysis is unknown, but it is probable that they injure the stroma by combining with or dissolving the lipoids. The addition of cholesterol and normal serum to saponin inhibits hemolysis, but compounds of saponin and lecithin are actively hemolytic. Their effects *in vivo* and *in vitro* have been studied extensively by Kobert.⁷

¹ Ztschr. f. Immunitätsf., 1912, 15, 506.

² Wien. klin. Wchn., 1904, No. 3; Münch. med. Wchn., 1904, No. 27.

³ Literature reviewed by Landsteiner, Handbuch der Biochemie des Menschen und der Tiere. Fischer, Jena, 1909, 444.

⁴ Münch. med. Wchn., 1905, 297.

⁵ Wien. klin. Wchn., 1905, 221.

⁶ Biochem. Ztschr., 1908, 12, 331; *ibid.*, 14, 275.

⁷ Archiv. f. exp. Path. u. Pharm., 1887, 23, 233. Also Die Saponinsubstanzen, Stuttgart, 1904.

McNeil¹ has applied the saponin hemolytic test to the study of red blood-corpuscles of human beings with various diseases. Resistance was greatly diminished in jaundice and the anemias, particularly in severe chronic jaundice and pernicious anemia. In diabetes mellitus and febrile conditions an increased resistance was frequently found. Neilson and Wheelon² have studied this reaction with particular care in relation to disease. With their method the average minimal degree of hemolysis of red blood-corpuscles in *whole* blood of healthy individuals was 1 : 13,937 sapotoxin solution, read after five minutes at 25° C. In cases of obstructive jaundice, pregnancy, pulmonary tuberculosis, lead-poisoning, and cardiorenal diseases an increased resistance was commonly observed; in hemolytic jaundice resistance was decreased, as likewise in the anemia of syphilis. In secondary anemias of carcinoma and tuberculosis and in pernicious anemia increased resistance was sometimes found. Washed corpuscles were more susceptible than corpuscles suspended in serum, and the cholesterol content of the blood appeared to run parallel to the degree of resistance offered by the cells.

In my experience saponin (Merck's) produces initial hemolysis of the citrated blood of healthy persons in dilution of about 1 : 30,000 according to the method described in this chapter; with washed corpuscles hemolysis occurs in dilutions above 1 : 40,000.

Hemolysis by Bacterial Poisons.—In the chapter on Toxins mention was made of the hemolytic poisons produced by many different bacteria, mostly pathogenic bacteria. Doubtless these hemotoxins are partly responsible for the production of the anemias developing during the course of many of the bacterial infections and notably those caused by streptococci and staphylococci.

The bacterial hemotoxins were first described by Koch,³ followed by the extensive studies of Ehrlich⁴ and Madsen⁵ upon the hemolytic poison produced by the tetanus bacillus (tetanolysin), and especially for the red blood-corpuscles of the horse and rabbit. Since then a very large literature has accumulated on this subject⁶ and the following bacteria are known to produce hemolytic poisons under favorable conditions:

Tetanus bacilli (tetanolysin).
 Staphylococci (staphylolysin).
 Streptococci (streptolysin).
 Pneumococci (endotoxin) (pneumotoxin).
 Many vibrios (vibriolysins).

Proteus bacillus (proteuslysin).

Bacillus megatherium (megatheriolysin).

Bacillus pestis (pestolysin).

Bacillus of malignant edema.

Colon, typhoid, and dysentery bacilli (cololysin, typholysin, dysenterolysin).

Bacillus pyocyaneus (doubtful) (pyocyanolysin).

Bacillus welchii.

These bacterial hemolysins are non-specific in that they will act upon the corpuscles of different animals, although some are affected more readily than others. Some are heat sensitive, and the immune sera of many are capable of neutralizing the hemolysins.

¹ Jour. Path. and Bacteriol., 1911, 15, 56.

² Jour. Lab. and Clin. Med., 1921, 6, 454, 487.

³ Berl. klin. Wchn., 1884, 477.

⁵ Ztschr. f. Hyg., 1899, 32, 214; *ibid.*, 32, 239.

⁴ Berl. klin. Wchn., 1898, 273.

⁶ Summarized by Landsteiner, Handbuch der Biochemie des Menschen und der Tiere, Fischer, Jena, 1909, 451.

The properties of these lysins are more or less characteristic of the micro-organisms producing them. Staphylolysin, for example, is destroyed by heating to 56° C. for twenty minutes, while streptolysin requires 70° C. for two hours, and typholysin is said to resist boiling. Connell and Holly¹ believe that streptolysin and megatheriolysin are specific fat complexes of the bacteria in definite colloid states.

Hemolysis by Animal Secretions.—Hemolytic poisons produced by spiders were observed in 1888 by Jousset and Sanarelli, and since then a large number of these poisons produced by spiders, various insects, fishes, and snakes have been studied. Especial attention has been given the hemolytic activity of cobra venom, first described by Stevens and Myers,² and since then by Myers,³ Stephens,⁴ Mitchell and Flexner,⁵ Flexner and Noguchi,⁶ McFarland and Weston,⁷ and many others.

The venoms appear to resemble the hemolysins to be found in the blood and may be activated by serum (complement) in much the same manner as the serum hemolysins. Cobra venom is most active, followed in order by the poisons of the water moccasin, copperhead, and rattlesnake.

The red blood-corpuscles of different animals vary in resistance to venom hemolysis, those of the dog being very susceptible. Among human beings the resistance varies in disease, being increased in the latter stages of syphilis, as shown by Weil,⁸ and after splenectomy, as observed by myself.⁹ This subject is considered in more detail in the chapter of Venom Hemolysis.

To this category of hemolytic substances belong the secretions of certain parasites, as *Bothriocephalus latus* and *Ankylostoma*; also the bile and pancreatic juice of mammals. These hemolytic substances have been generally identified with lipoids, as likewise the hemolytic activity of watery and salt solution extracts of tissues.

The hemotoxins in eel-serum are also to be classified in this category. This serum is extremely toxic and will kill the rabbit and guinea-pig when injected intravenously in amounts as small as 0.1 c.c. per kilo of body weight as shown by Mosso,¹⁰ Camus and Gley,¹¹ Kossel,¹² and others. Part of this toxicity is due to its remarkable hemolytic activity. The hemotoxin is thermolabile, being destroyed by heating to 55° C. for fifteen minutes; it resists the deteriorating influence of desiccation, but is easily destroyed by acids and alkalies. Animals may be immunized against this serum.

Hemolysis by Serum.—Just as bacteria may be killed and possibly broken up by specific bacteriolytic amboceptors and complements, so, in like manner, *hemolysis may be caused by specific hemolytic antibodies known as hemolysins*. Working in unison with complements the mechanism of both bacteriolysis and serum hemolysis is probably identical. The simplicity of hemolytic experiments, the rapidity with which they may be performed and terminated, and the ease with which hemolysis may be observed by the naked eye have rendered the specific serum hemolysins particularly useful in the study of amboceptors and of complements, and of cytolytic phenomena in general. In fact, bacteriolysis was not thoroughly understood until Bordet discovered the hemolysins, and demonstrated the analogy that exists between bacteriolysis and hemolysis, a discovery that led to a vast

¹ Jour. Bacteriology, 1921, 6, 89.

² Brit. Med. Jour., 1898, 621.

³ Jour. Path. and Bacteriol., 1899–1900, 415.

⁴ Jour. Path. and Bacteriol., 1899–1900, 273.

⁵ Nat. Acad. Sci., 1901.

⁶ Jour. Exper. Med., 1902, 3, 277.

⁷ Jour. Amer. Med. Assoc., 1909, 43, 845.

⁸ Jour. Infect. Dis., 1909, 6, 688.

⁹ Jour. Exper. Med., 1917, 25, 195.

¹⁰ Arch. Exp. Path., 1889, 25, 101.

¹¹ Compt. rend. Soc. d. biol., 1898, 126, 428

¹² Berl. klin. Wchn., 1898, 152.

amount of research work and controversy, to many important discoveries, and to the final evolvment of diagnostic reactions of great value.

SERUM HEMOLYSINS

Historic.—For many years physiologists were aware that the bloods of various animals transfused into man or animals of a different species were more or less directly injurious, and incapable of replacing human blood. In 1875 Landois demonstrated experimentally that while transfusion of blood from one animal to another of a different species may prove injurious and even fatal, transfusion to an animal of the same or of very closely related species produced no ill effects. The explanations offered were inadequate, until later researches on the hemolysins showed that the normal blood-serum of one animal may contain hemolysins for the erythrocytes of other animals, and, consequently, upon transfusing this blood to another animal the hemolysin acting with the complement present produced hemolysis *in vitro*, thereby explaining in part the toxicity of the alien blood.

In 1898 Belfanti and Carbone made the observation that the serum of a horse receiving several injections of rabbit blood was toxic for rabbits, whereas normal horse-serum was without injurious effects.

At about the same time Bordet published his epoch-making discoveries. He observed that while normal guinea-pig-serum had little or no hemolytic action on rabbit erythrocytes, the serum of a pig that had received a few intraperitoneal injections of rabbit blood was able quickly and completely to hemolyze rabbit blood, just as an animal may acquire, through immunization with cholera, the property of dissolving cholera vibrios. He demonstrated further that this acquired hemolytic activity was highly specific, for when animal A was immunized with the corpuscles of animal B the serum of A acquired the power of hemolyzing only the erythrocytes of B, and possibly of other animals closely related zoologically. It was found also that the hemolytic activity of an immune serum was lost by age or could be removed by heating; that in either case the serum could be reactivated by the addition of a little normal serum or peritoneal exudate—phenomena closely resembling that observed in bacteriolysis, and due to the action of a thermolabile body or alexin and a second and specific thermostabile antibody named by Brodet the “substance sensibilisatrice.”

These observations were soon confirmed by Landsteiner and von Dungern, and were followed by very extensive studies by Ehrlich and Morgenroth, who likewise confirmed Bordet's experiments, but offered a different explanation for the mechanism of the phenomenon, according to the side-chain theory, and renamed the alexin and sensitizing substance concerned in the process “complement” and “hemolytic amboceptor” or “immune body,” respectively.

Definition.—*Serum hemolysins* (Gr., αἷμα = blood + λύνειν = to dissolve) are antibodies in a serum that, when acting with complement, have the power of “lysing” or breaking up red blood-corpuscles, or so altering their envelope as to allow the hemoglobin to escape.

Nomenclature.—*Normal* or *natural hemolysins* are those found in normal serums; *specific* or *immune hemolysins* are those produced as the result of the injection of blood-corpuscles from an animal of a different species. *Heterolysins* are the hemolysins formed by immunization with corpuscles of a different species (the immune hemolysins). *Isolysins* are hemolysins for the corpuscles of animals of the same species. *Autolysins* are hemolysins that act upon the corpuscles of the same animal and are quite rare.

It should be remarked that isolysin and autolysin are not strictly synonymous terms, as the former does not act upon the corpuscles of the animal producing the hemolysin, but may hemolyze the corpuscles of other animals of the same species. For example, Ehrlich has shown that the serum of a goat that had received several injections of blood from other goats, although actively hemolytic for the corpuscles of goats 1, 2, 4, 5, 6, 9, and less so for goats 3 and 8, was not able to hemolyze those of goat 7 or of itself at all. This immunity of the corpuscles of an animal to its own isolysin was subsequently shown to be due to a complete absence of suitable receptors in its corpuscles. Therefore in cases in which a large internal hemorrhage occurs and the blood is absorbed an autohemolysin is not produced, or produced only in small amounts, and likely to be followed by the formation of an anti-autolysin which regulates the process of blood destruction within physiologic limits. Although little is known concerning the processes of normal blood destruction, as in the disposal of old erythrocytes, it is possible that an autohemolysin is being produced, and that its activity is held within normal limits by an anti-autolysin. A disturbance of this physiologic equilibrium may then be the basis of certain types of primary anemia characterized by excessive blood destruction.

The Nature of Hemolysinogens.—This refers to the nature or portion of erythrocytes concerned in the production of hemolysins when animals are injected with alien corpuscles.

A large amount of investigation has been devoted to this subject.¹ Bordet² has attributed the antigenic properties to the stroma of erythrocytes, while Nolf³ believes that this portion produces hemagglutinins and extracts of the cells, the hemolysin. Bradley and Sansum⁴ regard hemoglobin as antigenic, but Ford and Halsey⁵ were unable to produce hemolysins with purified hemoglobin; Levene⁶ likewise found that injections of pure crystalline hemoglobin failed to produce hemolysins, while solutions of erythrocytes in $\frac{1}{2}$ per cent. solutions of sodium carbonate were antigenic. It would appear, therefore, that some constituent of the erythrocyte other than hemoglobin is concerned in the production of hemolysins; the stroma are surely antigenic, and Bennett and Schmidt⁷ have recently found that a carbon dioxide globulin from washed erythrocytes or a substance intimately associated with it, is the antigen concerned in the production of hemolysins.

The chemical nature of the hemolysinogens is of greater importance and interest. According to Guerrini,⁸ the nucleoproteins of dog's erythrocytes are antigenic; Landsteiner and Javic,⁹ Eisler,¹⁰ Bang and Forssmann, and others, however, believe that the lipoids are antigenic. The latter found that ethereal extracts of red blood-corpuscles gave rise to the production of hemolysins, but not of agglutinins on immunization of animals. Others have failed to confirm these observations. Jobling and Bull¹¹ have found that the immunization of rabbits with hen corpuscles was followed by an increase of the serum lipases, and suggest that the lipoids may be

¹ Oppenheimer, *Handbuch der Biochemie des Menschen und der Tiere*, Fischer, Jena, 1909, 521.

² *Ann. de l'Inst. Pasteur*, 1898, 12, 688; 1899, 13, 225, 273; 1900, 14, 257.

³ *Ann. de l'Inst. Pasteur*, 1900, 14, 297, 656.

⁴ *Jour. Biol. Chem.*, 1914, 18, 497.

⁵ *Jour. Med. Research*, 1904, 11, 403.

⁶ *Jour. Med. Research*, 1904, 12, 191.

⁷ *Jour. Immunology*, 1919, 4, 29.

⁸ *Münch. med. Wchn.*, 1904, No. 27.

⁹ *Wien. klin. Wchn.*, 1904 676; *Centralbl. f. Bakteriöl.*, 1905, 39, 309.

¹⁰ *Centralbl. f. Bakteriöl.*, 1905, 40, 151.

¹¹ *Jour. Exper. Med.*, 1912, 16, 483.

antigenic. Vedder¹ was unable to produce hemolysins with ether extracts of corpuscles, or with globulin from stroma, but the protein extract left after the removal of globulin as well as lipoid-free stroma, produced hemolysin; essentially similar results were obtained by Chodat.² The consensus of opinion appears to be to the effect that protein-free lipoids are not antigenic, that the pure lipoids are not hemolysinogenic, and that the active antigenic substance is contained in the stroma of corpuscles.

Production of Hemolysins by Heterologous Antigens.—While antish sheep hemolysin, for example, is best prepared by injecting the rabbit or some other animal with sheep corpuscles, Forssman³ has demonstrated that this hemolysin could be prepared by immunizing rabbits with such heterologous antigens as extracts of the organs of the guinea-pig, horse, cat, dog, mouse, hen, pigeon, turtle, and even of some bacteria, as *Bacillus paratyphosus* B. Orndschiew⁴ has demonstrated similar heterologous antigens in serum, and Doerr and Pick,⁵ in urine. Doerr and Pick and Morgenroth⁶ found that when the organs of an animal contained an antigen for antish sheep hemolysin the corpuscles of this animal did not, and *vice versa*, but Kritchevsky⁷ found that nucleated corpuscles (hen) contained the hemolysin-producing antigen for sheep corpuscles just as the organs of the hen contain this substance. Kritchevsky also found that the connective tissue of guinea-pigs contain this antigen-producing hemolysin against sheep corpuscles.

As shown by Forssman and Hintze,⁸ the immune sera may also contain hemagglutinins for sheep corpuscles, as the result of immunization with heterologous antigens. These have been discussed in the chapter on Agglutinins.

These observations have not been adequately explained. The sera of the majority of rabbits show the presence of natural antish sheep hemolysin, and in my opinion the production of this hemolysin may be increased by non-specific stimulation by immunization with a variety of heterologous antigens, including not only those mentioned above, but even by certain chemical compounds, as arsphenamin and mercuric chlorid, as shown by the experiments of Toyama and myself.⁹ I have not been able to produce hemolysin for human corpuscles by injecting rabbits with the heterologous antigens mentioned, and natural antihuman hemolysin is rarely found in these animals.

The Nature of Serum Hemolysins.—The nature of these hemolysins is unknown. Evidently they are not ordinary chemical substances, since they do not act according to the laws of chemical equivalents and definite proportions. Many investigators have assumed that they were of the nature of lipolytic ferments; Bergel¹⁰ has stated that the sera of animals immunized to foreign erythrocytes are about twice as active in splitting foreign fats as the sera of untreated animals. Jobling and Bull¹¹ likewise found that the sera of rabbits immunized with hen corpuscles acquired an increased lipolytic action, and that a portion of lipases were specific for the corpuscles. Later lipases were more closely identified with the complements than with the hemolysins.

¹ Jour. Immunology, 1919, 4, 141.

² Compt. rend. Soc. de biol., 1921, 85, 735.

³ Biochem. Zeitschr., 1911, 37, 78.

⁴ Ztschr. f. Immunitätsf., 1912-13, 16, 268.

⁵ Biochem. Zeitschr., 1913, 1, 129; Ztschr. f. Immunitätsf., 1913, 19, 251, 612.

⁶ Berl. klin. Wchn., 1913, 1, 561.

⁷ Jour. Exper. Med., 1916, 24, 233.

⁸ Biochem. Zeitschr., 1912, xlv, 336.

⁹ Jour. Immunology, 1918, 3, 326

¹⁰ Deut. Arch. f. klin. Med., 1912, cvi, 47.

¹¹ Jour. Exper. Med., 1912, 16, 483.

Undoubtedly the serum lipases are concerned in the phenomenon of hemolysis, but appear to be more closely identified with the activity and nature of the complements than with the hemolysins. Investigations bearing upon the nature of the immunizing substances of erythrocytes discussed above indicate that the protein-free lipoids of these cells are not antigenic. The hemolysins produced by bacteria are regarded by Connell¹ and Holby² and others, however, as the bacterial fats in colloidal states. Hemolytic substances may be extracted from serum by ethyl alcohol and other fat solvents as shown by Woelfel³ and others, but these are not strictly specific. As discussed in the succeeding paragraphs, Metchnikoff has always regarded hemolysins as ferments derived from cells, but has failed to establish their identity and nature; they are commonly regarded as being identified with the globulin fraction of serum. At the present time, however, the hemolysins cannot be said to have been shown to be true ferments, proteolytic or lipolytic, although the process of serum hemolysis is apparently concerned with the action of serum lipases.

The Mechanism of Serum Hemolysis.—The side-chain theory of Ehrlich affords a working hypothesis upon the interaction of corpuscles, hemolysin, and complement as described below, but does not throw any light upon the nature and mechanism of serum hemolysis. Bordet believes that the hemolysin injures the stroma and prepares it for the destructive action of complement resulting in changing the resistance of the cell to osmotic influences, but as far as I know he has not offered an explanation of the mechanism of these changes.

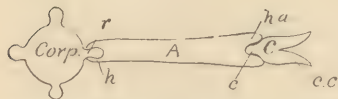


FIG. 126.—THEORETIC STRUCTURE OF A HEMOLYTIC AMBOCEPTOR (HEMOLYSIN).

A, Amboceptor; C, complement; r, receptor of the corpuscle; h, haptophore group of the amboceptor; h.a., complementophile group of the amboceptor.

The exact mechanism is not known and probably never will be until the nature of hemolysin has been determined. Undoubtedly the lipases of the serum and particularly of that portion designated as alexin or complement are concerned. Landsteiner, Neuberg, Bergel, and others regard hemolysis as a phenomenon due to the splitting or solution of the lipoids out of erythrocytes. Bergel⁴ has advanced the following hypothesis: the lipoids of erythrocytes are antigenic, and the hemolysins are specific zymogens formed by the lipoids of lymphocytes. The zymogens (hemolysins) are specifically bound by the lipoids of the corresponding corpuscles, which are then activated by the non-specific complement followed by solution of the lipoids of the corpuscles and hemolysis.

The Interaction of Hemolysin, Complement, and Corpuscles.—According to the side-chain theory, hemolysins are amboceptors or antibodies of the third order, requiring the action of a complement before hemolysis can be produced (Fig. 126). Bordet⁵ showed that two substances were concerned in the phenomenon of serum hemolysis, although his views on the mechanism of the process differ from those advanced by Ehrlich and Morgenroth.⁶

Theory of Ehrlich and Morgenroth.—Ehrlich argued that the hemolytic amboceptor or hemolysin must be an antibody to the receptors of the red blood-corpuscles used in the process of immunization, and if this is true, it ought to unite with the corpuscles. Taking the serum of a goat that had

¹ Jour. Exper. Med., 1913, 17, 61.

² Jour. Bacteriology, 1921, 6, 89.

³ Ann. d. l'Inst. Pasteur, 1899, 13, 273; 1901, 15, 312.

⁴ Berl. klin. Wchn., 1899, 6, 481.

⁵ Jour. Infect. Dis., 1905, 2, 97.

⁶ Ztschr. f. Immunitätsf., 1918, 27, 441.

been injected with and was hemolytic for the erythrocytes of a sheep, he destroyed the complement by heating the serum to 56° C. To this he added some sheep's corpuscles, and allowed the mixture to stand for a short time at room temperature, after which it was centrifuged and the supernatant fluid pipetted off in another test-tube. No hemolysis had occurred, and the corpuscles were to all appearances unaltered, but it was now found that if a small amount of normal goat-serum, as complement, was added to the corpuscles and the mixture placed in the incubator, hemolysis occurred. By adding sheep's corpuscles and normal goat-serum (complement) to the supernatant fluid that had been removed to a separate test-tube, hemolysis did not occur. This experiment indicated, therefore, that the red blood-corpuscles had combined with all the antibody. That the action was specific was shown by the fact that the corpuscles of other animals, such as rabbits or goats, for example, exerted no combining power when used instead of the sheep's cells. The union between cell and antibody was considered as being in the nature of a chemical combination and quite firm, as repeated washing of the cells with normal salt solution did not break it up.

Having shown that the antibody had a combining affinity for the cells, it was important to solve the question of the relation of alexin or complement to the process. In other words, does this substance unite directly with the cell or does it unite with the antibody and thus indirectly with the cell?

Ehrlich and Morgenroth studied this by means of a similar experiment. Sheep's corpuscles were mixed with normal goat-serum (complement), and after a time the mixture was centrifuged and the two portions tested separately. To the corpuscles heated immune serum was added, but hemolysis did not result. To the supernatant fluid corpuscles and heated immune serum were added and hemolysis occurred. This indicated that the alexin or complement did not unite with the corpuscles, as did the antibody in the first experiment, but remained free in the supernatant fluid.

By mixing corpuscles, immune serum, and complement, and keeping the mixture at 0° to 3° C. for several hours, hemolysis did not take place. By centrifuging the mixture and separating the supernatant fluid from the corpuscles, a similar test showed that the red cells had combined with all the antibody, but had left the alexin practically undisturbed:

At higher temperatures these relations were more difficult to demonstrate, as hemolysis occurs rapidly, but by leaving the cells and serum in contact for short periods of time, centrifuging rapidly, and testing the corpuscles and supernatant fluid in the same manner, similar relations were found to exist.

These experiments led Ehrlich to formulate the theory that complement will unite only with the antibody and not with the red corpuscles, but that it acts upon the corpuscles when united indirectly by means of the antibody. As will be pointed out further on, this view is in direct opposition to that of Bordet, who does not accept this interpretation, but believes that the complement acts directly upon the corpuscles.

Ehrlich, therefore, conceived the antibody as being in the nature of an amboceptor or of an interbody between an antigen and complement, with two combining arms—one the cytophil haptophore for union with the cell, and the second, the complementophil haptophore for union with complement. The amboceptor is unable in itself to injure the cell, but preserves its importance in being the only and specific means by which the ferment or complement can attack the cell and cause its destruction.

The process of specific serum hemolysis is, therefore, supposed to be

as follows: In fresh immune serum containing both amboceptors and complement, or in a mixture of old or heated immune serum and fresh normal serum (*i. e.*, of amboceptor and complement), the two substances occur independently of each other. When the corpuscles corresponding to the amboceptor are added, the amboceptor unites with these and the complement unites with the amboceptor, the amboceptor, therefore, standing midway between corpuscles and complement. When these unions have taken place, hemolysis will result. The amboceptor has a greater affinity for the corpuscles than the complement has for the amboceptor, and will unite with the cells at a low temperature, whereas the complement unites with the amboceptor only very slowly at low temperatures. Body temperature favors a quicker union of both, and especially that of complement with amboceptor. Hemolysis, therefore, may occur at low temperatures, but is hastened by higher temperatures, and occurs best at 37° C.

As stated in a previous chapter, Ehrlich believes that a great many complements exist in normal serums, which view is in direct opposition to the "unitarian theory" of Bordet, which holds that the one alexin or complement will act with any sensitizer or amboceptor. Of the large number of complements, each is especially adapted for the solution of one or more varieties of cells, which it can dissolve in conjunction with a suitable amboceptor. This is known as the main or dominant complement; other complements that may aid in the process are termed "non-dominant." In general, it is held that those complements that are especially active in hemolysis are but slightly active in bacteriolysis, and *vice versa*. Although the amboceptor is depicted as having but one haptophore arm for the dominant complement, it is really a polyceptor, and is so constituted that it combines with the cell to be dissolved, on the one hand, and with a number of complements, on the other.

Theory of Bordet.—Bordet has never accepted these views. He holds that the antibody is not an amboceptor for uniting cell and complement, but that it sensitizes the cell and renders it susceptible to the direct lytic action of the alexin or complement. According to his views, both antibody and complement may unite directly with the cell, and he has borne out this belief by making experiments almost exactly similar to those made by Ehrlich.

In accepting Ehrlich's view, it is a question of considerable practical importance whether the complement may unite directly with free amboceptor. Ehrlich maintains that the two may enter into a loose and easily dissociated chemical combination, which is hastened by heat and retarded by cold. The union of hemolytic amboceptor in cobra venom with the lecithin of corpuscles (Kyes' cobra lecithid), which acts as complement, while it tends to strengthen this view, can hardly be accepted as direct proof, as lecithin differs markedly from the ordinary complements found free in serum. Likewise, the theory of Neisser and Wechsberg regarding complement deviation, whereby it appears that an excess of amboceptor may combine directly with complement and in this manner rob those amboceptors that are attached to cells of the complement necessary to produce lysis, is quite complicated, and is not universally accepted, the evidence of direct union of complement and amboceptor having not been proved beyond the peradventure of a doubt. It follows, then, as Emery has stated, that we must either assume that the complementophil haptophore of an amboceptor united with its antigen has an increased affinity for complement over and above that of free amboceptors, or we must agree with Bordet that cell and complement unite directly after the former has been sensitized

by the action of the antibody. This latter view of the separate union of cell with antibody and complement is supported by the observations of Muir, who found, upon saturating red blood-corpuscles with antibody and then with complement, that some of the former, but none of the latter may be dissociated from the combination and become free in the fluid. However, the dissociated amboceptors found free of complement may be those that did not have time to unite with complement, and there does not appear to be any direct and positive experimental evidence to permit one to decide between Ehrlich's and Bordet's views.

While Ehrlich believes that the union between cell and amboceptor is a chemical one and follows ordinary chemical laws, obeying the law of multiple proportions, Bordet holds that the antibody acts as a mordant and sensitizes the cell, comparing the process to the staining of filter-paper when immersed in a dye, or to the use of mordants preparatory to the staining of flagella of certain bacteria. For example, 0.4 c.c. of a hemolytic serum, if added at once, was found to dissolve 0.5 c.c. of corpuscles. If 0.2 c.c. of corpuscles were first added, and amounts of 0.1 c.c. were added subsequently, no lysis took place after that of the first portion added. Bordet cited this as an example of a physical process of the nature of absorption, just as filter-paper when added at once to a dye will be stained a uniform color, whereas if it be added a little at a time, the first pieces inserted will be stained deeply, the subsequent ones less and less so, until the dye is completely absorbed. The process is probably not entirely a physical one as believed by Arrhenius,¹ who states that "the immune bodies are probably not bound by the erythrocytes, but only absorbed by them." *The phenomenon is greatly influenced by the concentration of the antibody in the serum, by the temperature at which corpuscles and hemolysins are brought together, by the length of exposure, reaction of the medium, and the amount of inorganic salts.* Ordinarily the union of corpuscles and hemolysins is quite rapid, fifteen minutes at 20° to 37° C. being usually sufficient, and Cromwell² states that absorption is more rapid with serum drawn early in the period of immunization. Recent researches on the colloidal theory of antibodies would indicate that the hemolysins are governed by very complex chemico-physical laws, not as yet fully understood, which regulate the action of colloids on one another and are probably intimately concerned in the processes under discussion.

Theory of Metchnikoff.—As was stated in a previous chapter, Metchnikoff maintained that both substances concerned in hemolysis are ferments, and that both are adapted for intracellular digestion. He regards complement or his cytase as a digestive ferment derived from leukocytes, and believes that it is set free only when leukocytes are dissolved (phagolysis) either as the result of the injection of a foreign substance or during the process of coagulation. Amboceptor or his "fixateur" is likened to enterokinase, and like it acts as an accessory ferment that unites the more potent ferment (cytase) to the particle to be digested. He also regards it as being derived from leukocytes, and considers that the amount formed depends upon the degree of phagocytosis that occurs during the absorption of the antigen. In the conception of immunity as being fundamentally a process of nutrition, and in the belief of the existence of more than one complement, the similarity between the views of Ehrlich and those of Metchnikoff is indeed striking.

Analogy Between Bacteriolysis and Hemolysis.—Studies in hemolysis aided greatly in a correct understanding of the mechanism of bacteriolysis.

¹ See *Immunochemistry*, MacMillan, 1907, 144, 257. ² *Jour. Immunology*, 1922, 7, 461.

It became apparent that two substances were concerned in bacteriolysis—one, the thermostabile amboceptor present in the immune serum, and the second, thermolabile alexin or complement, furnished by the peritoneal exudate in the Pfeiffer test, or by any fresh normal serum in the test-tube bacteriolytic reaction. The discovery and study of the specific serum hemolysins aided greatly in a better understanding of bacteriolysis and cytolysis in general.

Specificity of Hemolysins.—The hemolysins are highly specific antibodies, and although partial or group hemolysins may be formed and act upon the corpuscles of closely related species, as antihuman hemolysin on the corpuscles of the higher apes, yet the main hemolysin may be so potent that high dilution of the immune serum practically rules out the activities of group hemolysins, the hemolytic amboceptor proving highly specific for the alien corpuscles responsible for its production.

Distribution of Hemolysins.—Hemolysins are regularly found in the blood plasma as well as in the serum; the studies of Watanabe in my laboratory have shown that this is true of both natural and immune hemolysins.

When hemolysins are present in large amounts in the plasma they may occur in inflammatory exudates, but only exceptionally in transudates and such fluids as the *normal* cerebrospinal fluid and aqueous humors of the eye. In paresis, suppurative meningitis, and other conditions with increased permeability of the choroid plexus and meningeal hyperemia, hemolysins and, notably, natural antisheep hemolysin, may be found in the spinal fluid. Weil and Kafka have devised a test for this hemolysin in spinal fluid in connection with the diagnosis of these conditions.

Secretions rarely contain hemolysins unless the organ is markedly hyperemic, as colostrum and milk during the first few days before and after delivery. Urine is hemolytic due to acids and other substances, and only exceptionally contains specific hemolysins.

NORMAL OR NATURAL HEMOLYSINS

Just as small amounts of antitoxins, agglutinins, and opsonins may be found in normal serums, so also natural hemolysins may be present. Their most important practical significance is concerned with complement-fixation reactions, where a close and intimate quantitative relation exists between the complement and hemolytic amboceptor used. As an excess of hemolytic amboceptor may produce hemolysis with a decreased amount of complement in a given test for free complement, as in Wassermann's syphilis reaction, the patient's serum may contain so much natural antisheep amboceptor as to make up for slight binding of complement and give undue hemolysis or even a false negative result.

Hemolysis of alien corpuscles by a normal serum is found to depend upon the same mechanism of amboceptor and complement as in the artificial immune serums. The amboceptors are easily removed by adding the corresponding corpuscles to the cold serum and centrifuging the mixture after allowing it to stand at 0° to 5° C. for an hour or two. The supernatant fluid will now be found to be free from amboceptors, whereas by adding a little normal complement serum to the corpuscles hemolysis results, indicating that the amboceptors had been bound to these. If a normal serum contains several different amboceptors for as many different bloods, all may be removed at the one time by adding the respective corpuscles to the serum and allowing sufficient time to elapse for the amboceptors to become linked to their corpuscles.

Normal serum, therefore, probably contains numerous antibodies of the amboceptor type adapted to dissolve various foreign substances when they gain access to the blood. It is probable that, aside from hemolysins, various normal and immune cytolytins play an important part in the processes of immunity.

While the normal hemolysins that may be found in the serums of various animals have not as yet been fully worked out, the following table, compiled by Sachs,¹ is a résumé of the work reported in the literature on the subject:

NATURAL HEMOLYSINS

HEMOLYZE THE ERYTHROCYTES OF THE—	THE SERUM OF												
	Rabbit.	Guinea- pig.	Dog.	Man.	Goat	Ox.	Sheep.	Hog.	Horse.	Goose.	Duck.	Pigeon.	Hen.
Rabbit.	0	+	+	+	+	+	+	+	≠	+	+	+	+
Guinea-pig.	+	0	+	+	+	+	+	+	≠	+	+	≠	+
Dog.	(+)	≠	0										
Man.	≠	+	+	0	+	+	≠	≠	—	+	+	≠	+
Goat.	+	..	+	+	0	+	—	+	+	—	—
Ox.	≠	≠	≠	≠	..	0	—	≠	—	+	+	—	—
Sheep.	≠	+	+	+	≠	—	0	≠	—	—	—	—	—
Hog.	≠	+	..	—	—	0	—	+	+	—	+
Horse.	≠	+	+	+	..	+	≠	+	0	—	—	—	—
Goose.	+	—	—	—	—	0	—	—	—
Duck.	+	—	—	—	—	—	0	—	—
Pigeon.	+	+	..	≠	—	—	—	—	—	0	—
Hen.	+	+	—	—	—	—	—	—	0

+

= Well-marked hemolysis.

(+)

= Questionable or feeble hemolysis.

≠

= Doubtful.

—

= No hemolysis.

Kolmer and Casselman² have titrated the natural thermostabile hemolysins for the corpuscles of various vertebrates in a large number of human serums. Most interest centers about the occurrence of natural antish sheep hemolysin because of the wide-spread use of an antish sheep hemolytic system in complement-fixation reactions, as, for example, the Wassermann syphilis reaction. In over 80 per cent. of human serums there is present sufficient natural amboceptor for sheep's cells to give well-marked or complete hemolysis. Although this factor must be considered in using an antish sheep hemolytic system in complement-fixation reactions, yet with a proper understanding of principles and the employment of a satisfactory technic the danger of error is reduced to a minimum. In the following table the percentages of serums showing 100, 75, 25, and 0 per cent. hemolysis, with the maximum dose of serum (0.2 c.c.) used in the method of titration, are shown:

¹ Sachs, H.: Handbuch der pathogenen Mikroorganismen, Kolle and Wassermann, 2. Auflage, 2, p. 799.

² Jour. Infect. Dis., 1915, 16, 441.

SUMMARY OF NATURAL THERMOSTABLE HEMOLYSINS IN HUMAN SERUM

BLOOD.	NUMBER OF SERUMS TESTED.	PER CENT. SHOWING 100 PER CENT. HEMOLYSIS.	PER CENT. SHOWING 75 PER CENT. HEMOLYSIS.	PER CENT. SHOWING 25 PER CENT. HEMOLYSIS.	PER CENT. SHOWING NO HEMOLYSIS.
Sheep.....	125	64	20	9	7.5
Dog.....	25	16	36	30	18
Ox.....	85	6	20	24	50
Goat.....	25	0	8	16	76
Hog.....	40	0	1	3	96
Rat.....	25	0	0	12	88
Chicken.....	25	0	0	8	92
Horse.....	25	0	0	4	96
Rabbit.....	25	0	0	4	96
Guinea-pig.....	50	0	0	2	98

As discussed in section on the Properties of Hemolysins in this chapter, hemolysins are known to be thermolabile and thermostabile. Some of the natural hemolysins, and notably that for guinea-pig corpuscles in human sera, are almost entirely of the thermolabile variety, as shown in the following table by Kolmer, Trist, and Flick,¹ based upon the results observed in titrations employing unheated and heated human sera in amounts of 0.1 c.c.:

For erythrocytes of	Percentage sera thermo- labile hemolysin.	Percentage sera thermo- stable hemolysin.
Human.....	4 to 44	0 to 4
Sheep.....	85 to 95	80 to 95
Ox.....	72 to 88	5 to 30
Guinea-pig.....	90 to 100	20 to 25
Rabbit.....	94 to 100	0 to 2
Hog.....	92 to 100	0 to 15
Rat.....	4 to 32	0 to 2

The variable figures are due to the fact that *the natural hemolysins in human sera for the corpuscles of the lower animals are present in groups* analogous to the groups of the isohemolysins and isohemagglutinins in human sera. For this reason percentages expressing the presence of natural hemolysins based upon studies made with the corpuscles of a single animal of each species are only approximately correct.

Kolmer and Williams² have likewise studied the thermostabile hemolysins found in normal rabbit-serum as preliminary to some work concerning the site of formation of immune hemolysins. The results obtained with the maximum dose of serum (0.2 c.c.) are given in the following table:

SUMMARY OF NATURAL THERMOSTABLE HEMOLYSINS IN NORMAL RABBIT-SERUM

BLOOD.	NUMBER OF SERUMS TESTED.	PER CENT. SHOWING 100 PER CENT. HEMOLYSIS.	PER CENT. SHOWING 75 PER CENT. HEMOLYSIS.	PER CENT. SHOWING 25 PER CENT. HEMOLYSIS.	PER CENT. SHOWING NO HEMOLYSIS.
Goat.....	25	4	52	88	12
Sheep.....	50	18	56	76	24
Dog.....	25	32	40	72	28
Human.....	50	0	4	20	80
Hog.....	25	0	4	20	80
Ox.....	25	0	4	20	80
Chicken.....	25	0	4	8	92
Guinea-pig.....	25	0	0	4	96
Rat, white.....	25	0	0	0	100

¹ Amer. Jour. Syph., 1920, 4, 111.² Jour. Infect. Dis., 1913, xiii, No. 1, 96.

Of interest in this connection are the natural hemolysin and hemagglutinins in horse-sera for human corpuscles in relation to the therapeutic use of normal and immune horse-sera. Kolmer and Matsumoto¹ found agglutinins in practically all horse-sera for human corpuscles, but hemolysins are practically absent, being found only occasionally in fresh unheated serum and exceptionally in older and preserved sera.

Isohemolysins.—Hemolysins in sera for corpuscles of the same species are called isohemolysins; examples are hemolysins in human sera for human corpuscles and in guinea-pig-sera for guinea-pig corpuscles. These hemolysins, however, do not act upon the corpuscles of the individual from whom the serum is taken.

The isohemolysins are mostly thermolabile, as stated above; they are likewise highly susceptible to the effects of drying and cannot be well preserved in this state, as may the natural hemagglutinins.

Isohemolysins apparently occur in groups similar to the isohemagglutinins. This has been found true not only for the isohemolysins in human sera, but likewise for the isohemolysins in horse-, guinea-pig-, and the sera of other of the lower animals. The groups of isohemolysins for human corpuscles have not been studied as thoroughly as the isohemagglutinins, and it is not possible to state at present whether or not subgroups occur. For blood transfusion it is usual to conduct only agglutination tests for determining blood compatability, and for this reason the isohemolysins have not been studied as thoroughly as the agglutinins. I shall discuss their relation to blood transfusion in more detail in the section devoted to Transfusion.

As a general rule isohemolysins do not occur in human sera free of isohemagglutinins, so that bloods are usually tested for the latter only in preparation for transfusion. However, I am quite sure that isohemolysins may occur occasionally in sera in which hemagglutinins are not demonstrable.

Isohemolysins were apparently first discovered by Ehrlich and Morgenroth² by injecting a goat with an enormous amount of blood from other goats. The hemolysin was active for the corpuscles of other goats, but not for those of the immunized animal. Shortly afterward Ascoli³ discovered isohemolysins in human serum, and since then a large literature has accumulated, especially in relation to blood transfusion and the development of these substances in cancer and other diseases.

In 1892 Maragliano⁴ directed attention to the fact that the blood-serum of patients afflicted with various diseases exerted a hemolytic influence on the blood-corpuscles of healthy persons. Later Ascoli⁵ found the serums of cancer, pneumonia, and Addison's disease to be actively hemolytic for normal corpuscles. Kelling⁶ was first, however, to apply this test to the diagnosis of cancer. These reports stimulated many investigations, with the result that the presence of isohemolysins was regarded by many as a pathologic phenomenon. Weil⁷ early described the presence of hemolysins in the sera of dogs with a type of tumor known as infectious lymphosarcoma, active against the corpuscles of other dogs. Weil also observed, however, the presence of these hemolysins in normal dog-sera, but thought they underwent an increase in sarcomatous dogs, and particularly when the tumors showed necrosis. These hemolysins were not active against the dog's own corpuscles; indeed, it was believed that these cells acquired increased re-

¹ Jour. Immunology, 1920, 5, 75.

² Berl. klin. Wchn., 1900, 453.

³ Münch. med. Wchn., 1901, 1239.

⁴ Deutsch. med. Wchnschr., 1892, xviii, 411.

⁵ Münch. med. Wchn., 1901, xlviii, 1239.

⁶ Arch. f. klin. Chir., 1906, lxxx, 77.

⁷ Jour. Med. Research, 1907, 287.

sistance as the result of some sort of immunizing process by the tumor products.

Soon after this report Crile¹ published reports indicating the development of isohemolysins in the sera of human beings during the early stages of cancer and regarded their presence as possessing diagnostic value. Alessandri,² Janeway,³ Blumgarten,⁴ Scheiter,⁵ and others reported favorably upon the diagnostic value of the reaction, but Fischel,⁶ Whittemore,⁷ Smithies,⁸ Agazzi,⁹ Ottenberg,¹⁰ Gorliani and Lisser,¹¹ and others, were unable to confirm the diagnostic value of this isohemolysin reaction in cancer. Krida¹² has collected from literature 472 cases of cancer; of these, 67 per cent. gave positive reactions, while 2.6 per cent. of 509 observations on normal individuals yielded positive reactions.

These observations were made at a time when the importance of group isohemolysins in normal sera was not generally appreciated; however, it would appear that in some cases of cancer and particularly those associated with necroses and the accompanying cachexia, isohemolysins may be produced, but these changes do not occur early enough or with sufficient regularity to place a test for isohemolysins upon a diagnostic basis.

Autohemolysins; Paroxysmal Hemoglobinuria.—Hemolysins in the serum for the individual's own corpuscles are called autohemolysins. They are rarely observed and always in disease. Human autohemolysin was first discovered by Donath and Lansteiner¹³ and Eason¹⁴ in a case of paroxysmal hemoglobinuria, and these observations have since been generally confirmed. This hemotoxin or isohemolysin is capable of sensitizing the red blood-corpuscles of the patient or those of a normal person at a *low temperature*, hemolysis occurring in the presence of fresh serum, presumably with complement, and best at body temperature. As previously stated in the chapter on Agglutinins, autohemagglutinins have been demonstrated in the sera of cases of paroxysmal hemoglobinuria by Clough and Richter.¹⁵

According to Cook, about 90 per cent. of hemoglobinurics show a positive Wassermann reaction. Landsteiner found that about 10 per cent. of paretics showed the presence of isohemolysins, although these were not always autohemolysins. Malaria and trypanosomiasis have also been regarded as causes of paroxysmal hemoglobinuria, the most evidence, however, according to Matsuo¹⁶ and others, indicating that the disease usually has a syphilitic origin. Berghausen¹⁷ has suggested that the cold, trauma, and passive congestion leading to an attack are associated with the production of an excessive acidity of the tissues and that the organic acids thus formed may play some part in the hemolytic processes.

It is possible that the hemotoxin is similar to the hemolysin of cobra

¹ Jour. Amer. Med. Assoc., 1908, 40, 1883; *ibid.*, 41, 158.

² Jahresbericht f. Immunität., 1909, 5.

³ Jour. Amer. Med. Assoc., 1909, 43, 408.

⁴ Med. Record, 1909, lxxv, 61.

⁵ Jour. Amer. Med. Assoc., 1909, 43, 1481.

⁶ Berl. klin. Wchn., 1908, 882.

⁷ Boston Med. and Surg. Jour., 1909, clx, 77.

⁸ Med. Record, 1909, lxxv, 90.

⁹ Berl. klin. Wchn., 1910, No. 31.

¹⁰ Archiv. Int. Med., 1909, 3, 467.

¹¹ Amer. Jour. Med. Sci., 1912, 144, 103.

¹² Albany Med. Ann., 1911, 31, 259.

¹³ Münch. med. Wchn., 1904, 1590; Ztschr. f. klin. Med., 1906, 58, 173.

¹⁴ Edinb. Med. Jour., 1904, 19, 43.

¹⁵ Bull. Johns Hopkins Hosp., 1918, 29, 86.

¹⁶ Arch. f. klin. Med., 1912, 107, 335.

¹⁷ Arch. Int. Med., 1912, 9, 137.

venom, being in the nature of an amboceptor complemented by the fatty acids or lecithin of red corpuscles (endocomplement) or by a serum complement.

As previously stated, the hemolysin unites with the corpuscles at a low temperature, but hemolysis occurs only at higher temperatures. As shown by Meyer and Emmerich, and confirmed by Cook,¹ the complement also unites with the corpuscle-hemolysin complex at low temperatures, but, as just stated, higher temperatures are required for hemolysis. Persons with paroxysmal hemoglobinuria may have an attack merely by holding their hands in cold water. The autohemolysin will also sensitize the corpuscles of normal persons, as shown by Lorant² and others. Immediately after a paroxysm the hemolysin may be absent from the serum, as reported by Roberts.³

Moss⁴ has shown that the corpuscles of the patient may possess an increased resistance to hypotonic salt solutions, although just the reverse would be expected, and resistance to carbon dioxide is slightly increased, as shown by Berghausen.

Methods for conducting the autohemolysin test for paroxysmal hemoglobinuria are described in this chapter under Practical Applications. As shown by Meyer and Emmerich⁵ the test may fail owing to a lack of sufficient complement or owing to an anticomplementary action of the serum, as shown by Kumagai and Inoue.⁶

The Removal of Hemolysins from Serum by Absorption.—Natural or immune hemolysins may be removed from a serum by absorption with the homologous corpuscles. If the serum is active, that is, contains complement, it is first necessary to chill the serum to about 0° C. and add chilled corpuscles, the mixture being kept at 0° to 2° C. for several hours. At this temperature the hemolysin will unite with the corpuscles (sensitization occurs), but hemolysis does not occur or is very slight because complement is inactive at low temperatures. The mixture is now rapidly centrifuged; the supernatant fluid is found free of hemolysin under proper technical conditions when tested by adding complement and corpuscles, whereas the corpuscles are sensitized as found by adding complement and incubating at 38° C., when hemolysis occurs.

By first heating the serum at 55° C. for fifteen minutes the native complement is inactivated. Old sera need not be heated, as complement is destroyed in a few days unless extra precautions are taken. With inactivated sera the hemolysin may be removed by adding the corpuscles and keeping the mixture at room temperature for one-half hour or in a water-bath at 38° C. for fifteen minutes. At these higher temperatures sensitization is more rapid. The mixture is now centrifuged, when the supernatant fluid will be found free of hemolysin under proper technical conditions.

This process, however, *frequently renders a serum anticomplementary*, known as the "Sach's-Friedberger phenomenon."⁷ If absorbed sera are to be used in the Wassermann or other complement-fixation tests they should be reheated to 55° C. for fifteen minutes in order to remove the antilysins. Methods for the removal of hemolysins from active and inactivated sera are given under Practical Applications.

Absorption of sera with corpuscles does not remove syphilis "reagin"

¹ Ztschr. f. Immunitätsf., 1914, 21, 623.

² Deut. Archiv. klin. Med., 1918, 126, 148.

³ Brit. Med. Jour., 1915, 2, 398.

⁴ Bull. Johns Hopkins Hosp., 1911, 22, No. 245.

⁵ Deut. Arch. f. klin. Med., 1909, 96, 287.

⁶ Duetsch. med. Wchn., 1912, No. 8.

⁷ See Bauer, Deutsch. med. Wchn., 1908, 34, 698.

or antibodies other than the hemolysin corresponding to the corpuscles employed. Absorption with barium sulphate as originally advocated by Wechselsmann¹ has been found by Noguchi and Bronfenbrenner² to remove not only natural hemolysin, but syphilis antibody as well; these results were confirmed by Kytoku³ in my laboratory.

Natural Hemolysins in Relation to Complement-fixation Reactions.—Many investigators have expressed the opinion that the presence of natural antisheep hemolysin in human sera reduces the sensitiveness of the Wassermann and other complement-fixation tests conducted with an antisheep hemolytic system. Theoretically at least the presence of large amounts of natural hemolysin in some sera may be expected to disturb the important quantitative relations among corpuscles, complement, and hemolysin and thereby reduce the delicacy of complement-fixation tests. As shown by Williams,⁴ the same may occur with an antihuman hemolytic system due to iso-hemolysins, a fact commonly overlooked by those who employ this system for complement-fixation tests.

Practically, however, the subject is not of great importance, as the influence of natural hemolysins can be neutralized by simple technical procedures which will be discussed in more detail in the chapters devoted to the Complement-fixation Test. In my experience⁵ antisheep hemolysin is apt to be disturbing and reduce the sensitiveness of the Wassermann reaction only when present in large amounts in those sera containing small amounts of syphilis antibody.

Production of Hemolysins.—Because of their use in the serum diagnosis of syphilis and other infections hemolysins possess great practical value. They are best produced by injecting rabbits with washed human or sheep erythrocytes, or with those of some animal of another species. Rabbits differ considerably in their power to form hemolysins, and for some unknown reason hemolysins are more readily produced with some erythrocytes than with others. It is not possible, however, to immunize every kind of animal against every type of erythrocyte. As a general rule, an animal produces a better hemolysin the more remote its relationship is to the animal from which the erythrocytes for making the injection are taken.

Hemolysins may be prepared as the result either of intraperitoneal or of intravenous injections of erythrocytes washed at least three or four times to remove all traces of serum. Unless an antihuman hemolysin is required within a short space of time, better results are obtained, as a rule, by using the slower, intraperitoneal method for its production.

In immunizing rabbits it must be remembered that the quantity of hemolysin produced bears no direct relation to the size of the doses of corpuscles given. Thus, a highly potent hemolytic serum may be prepared by three intravenous injections of from 3 to 5 c.c. of a 10 per cent. suspension of washed sheep cells.

It must be emphasized that as far as possible an aseptic technic should be employed, and that corpuscles used for making intravenous injections should be filtered to remove small particles of fibrin, and preferably washed four times with sterile salt solution. The method of preparing corpuscles for injection has been given in Chapter II. After the third or fourth injection the animal should be bled from the ear and the serum tested, as animals not infrequently succumb after the fourth injection, and many

¹ Ztschr. f. Immunitätsf., 1909, 3, 528.

² Jour. Exper. Med., 1911, 13, 217.

³ Jour. Immunology, 1919, 4, 239.

⁴ Jour. Exper. Med., 1920, 32, 159.

⁵ Amer. Jour. Syph., 1920, 4, 135.

possess serums of high potency after receiving three injections. By this method success is better assured.

Production of Antihuman Hemolysin.—Noguchi and Bronfenbrenner¹ have found the rabbit best adapted for the preparation of antihuman hemolysin; more recently Sanford² has recommended the use of small dogs, claiming that these animals produce hemolysin equally well, but with the decided advantage of less coincident production of hemagglutinins.

It is difficult to prepare powerful antihuman and antichickem hemolytic sera; many rabbits succumb during the process of immunization. A variety of methods have been advocated, as follows:

Fatalities have been commonly ascribed to anaphylaxis, toxicity of human corpuscles for rabbits, agglutination, and hemolysis *in vivo*. The results of a study of these factors in my laboratory by Matsumoto³ indicated that primary and direct toxicity of human corpuscles for the rabbit was of most importance followed by agglutination and hemolysis *in vivo* and anaphylaxis. The toxic effects are most apparent when attempts are made to immunize rabbits with human, chicken, and guinea-pig corpuscles; sheep and beef corpuscles are much less toxic.

1. *Noguchi's Intravenous Method.*—Inject 4, 3, 4, and 3 c.c., and possibly another 4 c.c., with four- or five-day intervals; bleed nine or ten days after last injection.

2. *Noguchi's Intraperitoneal Method.*—Inject at four- or five-day intervals, bleeding nine or ten days after last injection. Doses 5, 8, 12, 15, and 20 c.c. of washed human corpuscles.

3. *Thompson's Method.*—Inject intravenously 0.1 c.c. washed cells every day for three or four weeks. This method was adopted after the work of Coca, showing that powerful hemolytic sera for sheep cells may be produced by this procedure.

4. *Craig's Method.*—Inject intravenously 1 c.c. of washed erythrocytes every other day until five or six injections have been given; two or three more injections may be required.

5. *Vedder's Method.*—Inject intravenously 0.5, 1, 2, and 3 c.c., washed and packed human cells at five to seven days, interval; the last injection of 3 c.c. may have to be repeated several times.

6. *Bronfenbrenner's and Schlesinger's Method.*—Rabbits are injected intravenously with washed red cells at four-day intervals; in order to avoid anaphylactic reactions each intravenous injection, beginning with the third one, is preceded by a desensitizing intraperitoneal injection of the same cells given one-half hour in advance.

In a comparative study of the above methods Miss Rule and the author⁴ found the intravenous methods superior to the intraperitoneal, inasmuch as hemolysin production occurred more quickly, required fewer injections, and smaller doses of cells. *Best results were secured in the production of antihuman, antichickem, antiguinea-pig, and antioox hemolysins by the daily intravenous injection of 0.1 c.c. washed packed cells diluted with 0.9 c.c. sterile saline solution, after the method of Thompson.*

Army Method.—I have also secured good antihuman hemolysins by the following method employed in the Army Medical School: Rabbits are given intravenous injections of 3 c.c. of packed, washed corpuscles on three successive days and are then allowed to rest for twenty-one days, when they are given daily intravenous injections of 0.5 c.c. of washed cells. If the titer is too low, a series of the 0.5 c.c. injections is repeated. A useful modification

¹ Jour. Exper. Med., 1911, 13, 78.

² Amer. Jour. Syph., 1920, 4, 697.

³ Jour. Immunology, 1920, 5, 507.

⁴ Amer. Jour. Syph., 1920, 4, 484.

of this method consists of three intravenous injections of 1 c.c. of a 50 per cent. suspension of washed cells at daily intervals, followed by three injections of 2 c.c. each daily and three more injections of 3 c.c. each daily. The animal is now rested for three weeks, when three injections of 3 c.c. each are given at daily intervals and the serum tested about one week after the last injection.

Sanford's Method.—Small dogs of the fox terrier size are recommended and given a series of intraperitoneal injections at weekly intervals of 50 per cent. suspensions of washed human corpuscles in doses of 30, 40, 50, 60, and 60 c.c.; sometimes a good hemolysin is secured with three injections.

Production of Antisheep Hemolysin.—The production of antisheep hemolysin in rabbits is so simple and easy as compared with the production of antihuman hemolysin that the subject has not attracted much attention; practically any method devised along generally accepted lines and with proper attention to the usual technical details will yield a satisfactory serum. Sheep cells are but slightly toxic for rabbits, and the antibody-producing tissues are promptly and powerfully influenced so that the majority of animals survive and produce highly potent immune sera. Agglutinin production for sheep cells is tardy and never reaches a high degree as compared with hemolysin production; no serologist of experience should consent to use any but highly potent sera in view of the ease of production, and no laboratory should be without the simple means of providing an adequate supply of this hemolysin.

Of the large number of methods advocated for the production of anti-sheep hemolysin, mention may be made of the following:

1. In the Hygienic Laboratory, Neill injects rabbits intravenously with 1 c.c. of fresh sheep cells every three days for four injections and tests the serum five days after the last injection; in a second method he injects intravenously 1, 1, and 2 c.c. washed cells on three successive days, with a second series of injections after an interval of five days, after the method of Gay and Fitzgerald.

2. Schweitzer and Stevens in the New York Department of Health Laboratories inject 0.25 c.c. of a 50 per cent. suspensions of washed cells every three days, increasing the dose by 0.25 c.c. with each injection until four to eight injections have been given.

3. Coca has advocated the daily administration of small doses (0.1 c.c.) of cells over a period of several weeks.

4. The author has used for several years an intravenous method consisting in the injection of 5 c.c. of a 10 per cent. suspension of washed cells every three days until four to six injections have been given. This method is very simple and has uniformly yielded powerful antisheep hemolysin.

Intraperitoneal injections may also be used, consisting in the injection of 5 c.c. of 50 per cent. suspensions of washed corpuscles every five days until four to six injections have been given.

The Collection of Serum.—Most authors advise bleeding an animal four to nine days after the last injection of cells when preliminary titrations conducted with serum secured by collecting blood from an ear vein have shown that the titer is satisfactory. According to our experiments *the best time for bleeding appears to be about seven days after the last injection by the intravenous route*, inasmuch as hemolysin production is likely to occur for about this time after an injection of the antigen.

A preliminary titration of serum secured from a small amount of blood from an ear vein may not give an exact idea of the content in hemolysin,

inasmuch as the serum of the whole blood secured by bleeding the animal to death may show less or slightly more hemolysin.

Preservation of Serum.—Methods have been described in Chapter IV. I have found that the addition of an equal part of chemically pure glycerin to unheated serum constitutes an excellent method. Antihuman serum is also well preserved dried in filter-paper, as described by Noguchi.

As previously stated, the portion of the erythrocyte that is responsible for the production of hemolysins is a moot question. Bordet and von Dungern maintain that the stroma is the exciting agent; Nolf and others believe that the stromata produce hemagglutinins, and that the hemoglobin is chiefly concerned in the production of the hemolysin.

General Properties of Hemolysins; Thermostabile and Thermolabile Hemolysins.—Immune hemolysins are highly resistant antibodies, and are easily preserved. Sterile immune serum may be inactivated by heating in a water-bath for half an hour at 56° C., and may be preserved for many months if small amounts are placed in ampules and kept in a cold place. If an equal quantity of neutral glycerin is added to the clear inactivated serum it will aid greatly in its preservation. The immune hemolysins resist drying to a well-marked degree, and filter-paper saturated with the serum and dried, after the method of Noguchi, preserves the hemolytic activity in a remarkable degree. Heating an immune serum at 56° C., for half an hour, as in the process of inactivating complement, does not materially injure the hemolytic activity of a potent serum. A temperature of 70° C. or above may cause deterioration and finally destroy the hemolysins.

A portion of immune hemolysin, however, is thermolabile and easily destroyed by heating serum to 55° C. for half an hour. Many of the natural hemolysins and especially those in human serum for human corpuscles and the corpuscles of the guinea-pig, rabbit, hog, and ox are of the thermolabile variety.

Thiele and Embleton¹ have sought to prove that these thermolabile hemolysins are only differentiated complements, that is, immune hemolysin as it is produced is first thermolabile and gradually acquires thermostability as production is continued. Sherman,² on the other hand, regards all hemolysins as thermostabile and that the reduction in hemolytic activity of a serum as a result of heating is due to "masking" of the hemolysin rather than an actual destruction or inactivation, that is, the hemolysins are incapable of sensitizing corpuscles in the presence of complement. In my own experiments, however, I found that the thermolabile hemolysins may be differentiated from the complements, being more resistant to heat; in this respect my results do not agree with those of Thiele and Embleton. On the other hand, these experiments have indicated that heat may actually destroy some of these hemolysins, that for sheep cells being more resistant than that for guinea-pig corpuscles.³

While immune hemolysins are largely resistant to the deteriorating influence of desiccation, the natural hemolysins are more susceptible. I have found that the isohemolysins in human sera deteriorate rapidly upon drying, and under ordinary conditions are more susceptible than the isohemagglutinins.⁴

As stated in the preceding chapter, the hemolysins have been generally found in the englobulin and pseudoglobulin fractions of the serum, and not in the albumin fraction, by Fuhrmann⁵ and others.

¹ Ztschr. f. Immunitätsf., 1914, 20, 1.

² Jour. Infect. Dis., 1918, 22, 534.

³ Jour. Immunology, 1919, 4, 403.

⁴ Jour. Immunology, 1919, 4, 393.

⁵ Hofmeister's Beitr., 1903, 3, 417.

As was previously mentioned, hemolytic amboceptors possess a great affinity for the receptors of their homologous corpuscles, and will readily unite with them at a low temperature. At incubator temperature the union is quite rapid, so that corpuscles may be "sensitized" within half an hour.

Source of Hemolysins.—As has been stated elsewhere, Metchnikoff regards the leukocytes as the source of fixateur or amboceptor formation. Recently Carrel and Ebeling¹ have furnished direct experimental evidence of leukocytes as a possible source of hemolysin production. These investigators have found that cultures of leukocytes in hen plasma elaborate a hemolytic substance for rabbit and sheep erythrocytes in about 50 per cent. of cultures. Bullock found that the amount of hemolytic amboceptor in a serum runs parallel with the number of mononuclear leukocytes, and he regards this as an indication of the activity of the lymphoid tissue in general, which he considers as the main source of amboceptor formation. While it is probable that endothelial cells and mononuclear leukocytes are especially concerned in the process, our own investigations in this field would indicate that the process is more general, being participated in by cells of other tissues which possess suitable combining affinities for the alien corpuscles.

Antihemolysins.—A further step in the study of hemolysins, but one more of theoretic than of practical interest, was the discovery of antihemolysins. Besredka² first discovered these antihemolysins in normal sera. By injecting guinea-pigs with normal rabbit-serum containing amboceptors for ox blood Bordet³ secured a serum that inhibited the action of anti-ox immune serum. Ehrlich and Sacks,⁴ by injecting a goat with normal rabbit-serum, likewise secured a serum that acted as an anti-amboceptor against immune hemolytic amboceptors for ox blood. Ehrlich argued that the anti-amboceptor acted against the complementophil group of the amboceptor, which prevented union with a complement from taking place. This view was advanced in support of his theory concerning the two-armed character of the amboceptor and that an anti-amboceptor may be produced against either the cytophil or the complementophil group, or both. In these particular serums, however, the investigators may have been working with an anticomplement instead of an anti-amboceptor.

A specific hemolysin—one, for example, specific for dog blood, derived from treating a rabbit with dog cells—is highly toxic for dogs, being capable of producing hemolysis *in vitro* and a clinical condition known as hemolytic jaundice. It is possible, however, gradually to immunize a dog against this amboceptor for his own cells by starting with very small doses and gradually increasing these until it is found that the animal tolerates amounts that would be fatal to non-immunized animals. If a portion of this serum is now added to the specific hemolytic serum, it will be found that the power of the latter is inhibited. Although this action may likewise be due to anticomplement, it is probable that an anti-amboceptor against the cytophil group of the amboceptor is also formed, which prevents the amboceptor from uniting with the red blood-cells, although conclusive experimental evidence of this has not been adduced.

The Protective or Antihemolytic Activity of Sera; Relation to Anemia.—While sera may be hemolytic for the corpuscles of various animals, depending upon the presence of natural hemolysins and complements, it is now well known that normal serum may be antihemolytic for various hemo-

¹ Jour. Exper. Med., 1922, 36, 645.

² Ann. d. l'Inst. Pasteur, 1901, 15, 785.

³ Ann. d. l'Inst. Pasteur, 1899, 13, 273; 1900, 14, 257.

⁴ Berl. klin. Wchn., 1900, 681; 1901, 569.

toxins. Müller¹ showed that heated serum may prevent hemolysis by serum hemolysins. Neisser and Friedemann,² Bergmann and Keuthe,³ and others have noted this property in human sera, and Marshall and Morgenroth⁴ have attributed the effects to a destructive effect upon complement. For this reason the process has been designated as the *anticomplementary activity of sera* and is of great practical importance in relation to the reaction of complement fixation; the phenomenon will be discussed in greater detail in the chapters devoted to that subject.

In addition to this anticomplementary action, normal sera may protect corpuscles to a marked degree against the hemolytic activity of saponin, bacterial hemotoxins, sodium oleate, lactic acid, and other substances. Ransom⁵ has expressed the opinion that this neutralizing effect is due to the presence of cholesterol in serum and corpuscles in so far at least as saponin hemolysis is concerned. Bruere,⁶ however, was unable to confirm this opinion. Goldberger⁷ found that the sera of cancerous persons acquired an increased antihemolytic activity over oleic and lactic acids, but Sweek and Fleischer⁸ did not observe that these sera were any more antihemolytic than the sera of normal individuals. Clark and Evans⁹ found the sera of normal persons and of patients with diseases without anemia remarkably constant in antihemolytic activity for sodium oleate on guinea-pig corpuscles; in anemias, however, and especially in pernicious anemia and conditions associated with lesions of the spleen, a marked reduction in protective power was found. The authors have suggested that this reduction may constitute a factor in the mechanism of the so-called hemolytic anemias, Addisonian anemia, and hemolytic jaundice. Subsequent studies on the nature of the antihemolytic substances in serum for sodium oleate and saponin have shown that this property does not depend alone upon the serum cholesterol, lecithin, or a combination of these, and the diminished protective power associated with the above-mentioned anemias cannot be explained simply on the basis of the diminution of the total cholesterol.

Hemolysins in Relation to Complement Fixation.—There is probably no other group of antibodies that possesses greater diagnostic value than do the hemolysins, a fact that was demonstrated in the practical application of the Bordet-Gengou phenomenon of complement fixation in the diagnosis of syphilis and other infections. By adding sensitized corpuscles—*i. e.*, corpuscles with their homologous amboceptors—to a fluid, the presence or absence of complement may be determined. If complement is present, hemolysis will occur and be complete or partial, depending upon the amount of complement available; if hemolysis does not occur, it may be concluded that free complement is absent. This is the basis of the complement-fixation diagnosis of syphilis, gonorrhea, glanders, differentiation of proteins, etc. When a proper amount of complement is added to a mixture of antigen and its immune serum (containing amboceptors), it is bound to these amboceptors, so that when corpuscles and hemolytic amboceptor are subsequently added, hemolysis does not occur, since there is no available complement, it having been “fixed” by the first amboceptors. If, however,

¹ Centralbl. f. Bakteriöl., 1901, 29, 860.

² Berl. klin. Wchn., 1902, 677.

³ Ztschr. f. Exp. Path., 1906, 3, 255.

⁴ Deutsch. med. Wchn., 1901, March 28th.

⁵ Ibid.

⁶ Jour. Med. Research, 1902, 8, 362.

⁷ Folia Serologica, 1911, 7, 941.

⁸ Jour. Med. Research, 1913, 27, 383.

⁹ Bull. Johns Hopkins Hosp., 1920, 31, 354; 1921, 32, 113, 328.

amboceptors for the antigen in the first instance are not present, as where a normal serum is used, the complement remains free and acts with the hemolytic amboceptor to produce hemolysis of the test corpuscles. In this manner the hemolysins and their corresponding corpuscles are employed as indicators or tests for the presence of free complement, so that if an antigen is known, the antibody may be determined; or vice versa, by using a known antibody, the antigen may be determined, the criterion in each instance being whether complement is or is not bound or "fixed," a fact that is determined by the subsequent addition of a hemolysin and its homologous corpuscles.

The practical applications and technic of these reactions are given in subsequent chapters.

Quantitative Relationship Between Hemolysin, Corpuscles, and Complement.—From a practical as well as a theoretic standpoint an important property of hemolysin and of complement is the quantitative relationship that each bears to the other. This is especially important in hemolytic reactions, where an excess of either may compensate for a decrease of the other and yield fallacious results. If a certain amount of guinea-pig's complement is necessary to lyse 1 c.c. of a 2.5 per cent. suspension of sheep's cells, along with hemolytic amboceptor, then double this amount of complement will be required to lyse 2 c.c. of the same blood, and so on. If a constant quantity of corpuscles and hemolysin are added to a series of test-tubes, and increasing amounts of complement, after incubating the mixtures for an hour the smallest amount of complement that produces complete hemolysis is called a *unit*, and in this manner the strength or activity of a serum complement is measured or titrated. In a similar manner the hemolytic activity of a serum or its measure of hemolysin may be determined by placing in a series of tubes, as previously directed, a definite and equal amount of corpuscle suspension, and to each tube is then added an amount, also definite and equal, of a normal serum as complement; there are next added decreasing and graduated amounts of the immune serum whose native complement has been destroyed by inactivation. After incubating the mixtures for an hour, the smallest amount of inactivated immune serum that will just produce complete hemolysis is known as the *amboceptor* or *hemolysin unit* of the serum. In other words, there are three substances concerned in serum hemolysis: the hemolysin, the corpuscles, and the complement. By taking two of these as constants, *e. g.*, the corpuscles and the complement, the unit of hemolysin may be determined; or by taking the corpuscles and hemolysin as constants the unit of complement may be determined. Since the corpuscles and hemolysin are most stable, these may be used as constants, and the unit of complement determined under these conditions as preliminary to complement-fixation reactions.

It is important to bear in mind, in this connection, that the titer of an immune hemolytic serum will vary with the complement used. For example, an antish sheep hemolysin is much more active when guinea-pig serum is used as complement than it is when tested with the same quantity of rabbit-serum as complement.

After determining the unit of hemolysin or complement—that is, after adjusting the hemolytic system to exact proportions—the results that follow the varying quantities of complement and hemolysin require the most careful consideration. Less than one unit of amboceptor with one unit of complement cannot yield complete hemolysis; likewise if with one unit of hemolysin less than a unit of complement is combined hemolysis is incomplete; with one unit of hemolysin and one unit of complement

and a double dose of corpuscles hemolysis will also be incomplete. With less than a unit of complement and an excess of hemolysin, however, hemolysis may be complete. The complement may be reduced to so small an amount that hemolysis is incomplete no matter how much hemolysin is used, but the important fact to be borne in mind is that a slight decrease in complement may be compensated for by the presence of many units of hemolysin, so that complete hemolysis results and a false reaction is secured. The converse of this is true to a less marked extent—*i. e.*, an excess of complement may compensate for a decrease in hemolysin, but is less capable of doing so.

These facts are of the utmost importance in making hemolytic experiments, as in complement-fixation reactions, where the entire test depends upon demonstrating whether or not a portion or the whole of the complement used has been fixed. Unless, in a series of hemolytic reactions, the amount of hemolysin employed is the same throughout, the amount of complement acting in these cannot be determined by comparing the degree of hemolysis. This is true especially in cases where a small amount of complement is fixed, as in the Wassermann reaction, with a serum containing a small amount of syphilitic antibody, when the presence of an excess of hemolytic amboceptor may give complete hemolysis and overshadow the fact that a small amount of complement has been actually fixed by syphilitic antibody and antigen.

PRACTICAL APPLICATIONS

Method of Titration of Immune Hemolysin.—Various methods have been employed by different workers in this field, but all are based upon the same principles as have been here outlined.

A small amount of immune serum is inactivated by heating in a water-bath at 56° C. for half an hour. In testing the serum of a rabbit during the process of immunization 2 or 3 c.c. of blood are easily secured from the ear, and the serum is separated. After it has been inactivated the serum is diluted to 1 : 100 (1 c.c. of serum of 99 c.c. of salt solution, or 0.1 c.c. serum + 9.9 c.c. of salt solution).

Fresh guinea-pig serum is secured for complement by bleeding a healthy pig under ether anesthesia into a Petri dish or centrifuge tube. This serum is diluted 1 : 20, making a 5 per cent. solution, by adding 1 c.c. of serum to 19 c.c. of salt solution. Each cubic centimeter of this dilution contains 0.05 c.c. of undiluted serum, which experience has shown is a satisfactory amount to use.

The corpuscle suspension is then prepared. The blood used depends upon the kind of hemolysin that is to be titrated. With sheep and ox blood, a 2.5 per cent. suspension of washed corpuscles may be employed. With antihuman hemolysin, the corpuscles are usually used in 1 per cent. suspension. (See Noguchi Modification of Wassermann Reaction.) After the corpuscles have been washed three times, 1 c.c. is placed in 39 c.c. of salt solution, or sufficient salt solution is added to 2.5 c.c. of the corpuscles to make the total volume equal 100 c.c.

To a series of six sterile test-tubes increasing doses of the diluted immune serum are now added, together with 1 c.c. of complement dilution, 1 c.c. of corpuscles suspension, and sufficient normal salt solution to make the total volume in each tube about 3 or 4 c.c. The following table shows the method of preliminary titration of a hemolytic serum:

PRELIMINARY TITRATION OF A HEMOLYSIN

TUBE.	AMOUNT OF INACTIVATED IMMUNE SERUM IN C.C. (1 : 100).	DOSE OF COMPLEMENT, C.C. (1 : 20).	DOSE OF CORPUSCLES, C.C. (2.5 PER CENT.).	NORMAL SALT SOLUTION.	RESULT OF HEMOLYSIS AFTER ONE HOUR IN THE WATER-BATH (37° C.).
1.....	0.1 (0.001 c.c. undiluted)	1	1	q. s. 3 c.c.	No hemolysis.
2.....	0.2 (0.002 c.c. undiluted)	1	1	q. s. 3 c.c.	Partial hemolysis.
3.....	0.4 (0.004 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
4.....	0.6 (0.006 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
5.....	0.8 (0.008 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
6.....	1.0 (0.01 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.

In this instance the unit of amboceptor is 1 : 250, which is too low for a satisfactory antisherp serum. The rabbit should, therefore, receive another dose or two of corpuscles, and the serum be titrated again in from four to seven days after the last injection has been given. In this titration it will be well to use a higher dilution of the inactivated immune serum, as 1 : 1000. This may be prepared by adding 1 c.c. of a dilution of 1 : 100 with 9 c.c. of normal salt solution and mixing well. The titration is then proceeded with as follows:

TITRATION OF HEMOLYSIN

TUBE.	AMOUNT OF INACTIVATED IMMUNE SERUM IN C.C. (1 : 1000).	DOSE OF COMPLEMENT IN C.C. (1 : 20).	DOSE OF CORPUSCLES, C.C. (2.5 PER CENT.).	NORMAL SALT SOLUTION.	RESULT OF HEMOLYSIS AFTER ONE HOUR IN THE WATER-BATH AT 37° C.
1....	0.05 (0.00005 c.c. undiluted)	1	1	q. s. 3 c.c.	No hemolysis.
2....	0.1 (0.0001 c.c. undiluted)	1	1	q. s. 3 c.c.	No hemolysis.
3....	0.15 (0.00015 c.c. undiluted)	1	1	q. s. 3 c.c.	Beginning hemolysis.
4....	0.2 (0.0002 c.c. undiluted)	1	1	q. s. 3 c.c.	Partial hemolysis.
5....	0.25 (0.00025 c.c. undiluted)	1	1	q. s. 3 c.c.	Just complete hemolysis.
6....	0.3 (0.0003 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
7....	0.35 (0.00035 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
8....	0.4 (0.0004 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
9....	0.45 (0.00045 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.
10....	0.5 (0.0005 c.c. undiluted)	1	1	q. s. 3 c.c.	Complete hemolysis.

The following controls should be set up at the same time:

- 1 c.c. of corpuscles in 1 c.c. of amboceptor dilution. This tube should show no hemolysis, as the serum has been inactivated and is too highly diluted for complement activity, even though native complement were present.
- 1 c.c. of corpuscles in 1 c.c. of complement dilution. This tube may show a trace of hemolysis, due to the presence of a small amount of natural hemolysin for the corpuscles used. As a general rule, guinea-pig serum is free from natural antisherp hemolysin, or the amount is so small under these conditions that it is not necessary to remove it.
- 1 c.c. of corpuscles in 3 c.c. of salt solution. This tube should show no hemolysis, and serves to show that the diluent was isotonic.



FIG. 127.—TITRATION OF HEMOLYTIC AMBOCEPTOR.

The tube containing 0.3 c.c. hemolytic amboceptor is the smallest amount showing complete hemolysis, and this amount is the *unit*.

In the foregoing titration it is found that 0.25 c.c. of 1 : 1000 dilution of amboceptor is the unit, or the titer is 1 : 4000. The method requires accurate pipets and careful work, but yields uniform and satisfactory results (Fig. 127).

This method is given only as an example of the technic and principles involved. The method of titration varies according to the hemolytic system being employed for conducting complement-fixation tests, and will be referred to again in the chapter devoted to this technic.

Instead of using varying amounts of a stock solution of hemolysin as described, a series of dilutions of hemolysin may be prepared, as 1 : 1000, 1 : 2000, 1 : 3000, etc., and employed in a constant dose of 0.5 or 1.0 c.c.

If the serum is satisfactory the rabbit may now be bled under anesthesia. The serum is separated and inactivated and again titrated, as the final titration, for some unknown reason, is likely to be a little lower than in the primary tests.

When suitably preserved, a hemolytic serum will maintain its activity for long periods of time; it should always, however, be titrated before complement-fixation tests are undertaken.

Methods for Removing Hemolysins from a Serum.—In general, these aim to remove the natural hemolysins, such as natural antisheep hemolysin, from human serums preliminary to making the Wassermann test, or from a guinea-pig-serum that is to be used as complement.

The method of removal consists simply in adding corpuscles to the serum, and allowing sufficient time for the corresponding hemolytic amboceptor to become attached, and then removing both by centrifuging the mixture. If the serum is fresh, it should be cooled to 0° to 3° C. in order to inhibit complement activity, which would hemolyze a portion of the corpuscles.

Method for Removal of Natural Hemolysins from Active Serum.—(1) Chill the serum in a mixture of ice and salt; (2) for each cubic centimeter add 1 drop of sediment of washed corpuscles thoroughly chilled; (3) mix and keep at or near 0° to 2° C. for at least one hour; (4) centrifuge rapidly and preferably with the tube surrounded with ice. Remove the supernatant serum.

In removing a natural hemolytic amboceptor from a guinea-pig-serum that is to be used as complement a measured amount of serum is first removed to a separate tube and thoroughly chilled in a glass of cracked ice. If a large amount of serum is to be used, for example, 5 c.c., it is well to place about 0.1 to 0.2 c.c. of pure undiluted corpuscles, after their last washing, in the bottom of a centrifuge tube. This quantity of corpuscles does not materially affect the dilution of the serum. If a smaller amount of serum is used, such as 1 c.c., it is well to add 8 c.c. of a 2.5 per cent. suspension of chilled corpuscles, and after keeping the mixture at or near 0° to 2° C. for an hour, centrifuging the mixture; the final dilution of 1 : 20 is secured by adding 10 c.c. of salt solution to the supernatant diluted serum.

Method of Removal of Natural Hemolysins from Inactivated Serum.—This method refers especially to the removal of natural antisheep hemolysin from human sera for the Wassermann and other complement-fixation tests. (1) Heat the serum in a water-bath to 55° C. for ten minutes. This suffices for the inactivation of complement. (2) For approximately each cubic centimeter of serum add 1 drop of sediment of washed corpuscles. Mix and stand at room temperature for about half an hour or in a water-bath or thermostat at 38° C. for fifteen minutes. (3) Centrifuge and remove the

supernatant serum. (4) Heat the sera to 55° C. for fifteen minutes to remove anticomplementary substances.

The time allowed for absorption of hemolysin may be less than stated unless a serum contains relatively large amounts of hemolysin. Kahn¹ uses a period of ten minutes; Simon² has advocated a method employing ten minutes in a water-bath at 37° to 40° C.

Method of Determining Natural Hemolysins in Serum.—To ascertain whether or not a certain natural hemolysin is present in a serum it is merely necessary to inactivate the serum, and to a measured amount, for example, 0.2 c.c., add 1 c.c. of complement serum (1 : 20) that is known to be free of the particular hemolysin in question, and 1 c.c. of a 2.5 per cent. suspension of the corresponding corpuscles. Sufficient salt solution is added to bring the total volume to 3 or 4 c.c. The mixture is then incubated at 37° C. for one or two hours, when the occurrence of hemolysis indicates the presence of the hemolysin for the corpuscles employed.

If the serum is fresh the native complement may be employed. In this case the test is conducted by placing 0.1 or 0.2 c.c. serum in a test-tube with 1 c.c. of suspension of washed corpuscles homologous with the hemolysin being sought, followed by incubation in a water-bath at 38° C. for one hour, when the presence or absence of hemolysis will be evident.

To determine the amount of natural hemolysin by titration dilute the serum with 9 parts of normal salt solution, and to a series of test-tubes add increasing amounts of 0.05 c.c., 0.1 c.c., 0.2 c.c., 0.4 c.c., 0.8 c.c., 1 c.c., and 2 c.c., corresponding respectively to 0.005, 0.01, 0.02, 0.04, 0.08, 0.1, and 0.2 c.c. of the undiluted serum. Add 1 c.c. of a 5 per cent. dilution of fresh hemolysin-free guinea-pig serum as complement, and 1 c.c. of a 2.5 per cent. suspension of the corpuscles; sufficient salt solution is added to make the total volume about 3 c.c. After shaking, the tubes are placed in the incubator at 37° C. for one hour, removed, and the results read, or the tubes may be placed in a refrigerator overnight and the results read in the morning.

SERUM DIAGNOSIS OF PAROXYSMAL HEMOGLOBINURIA

First Method.—According to Ehrlich, a small tourniquet should be applied about the base of one of the patient's fingers, and this is then kept immersed in ice-cold water for half an hour. Blood from the finger thus constricted is then collected in a Wright capsule, and blood from a finger of the other hand is used as a control. Both are allowed to clot and are then centrifugalized. The serum from the finger held in iced water is tinged red from dissolved hemoglobin, whereas the control serum is not tinged or at least not tinged so deeply.

Second Method.—Donath and Landsteiner have applied Ehrlich's method *in vitro*. Their method consists of collecting blood in a small test-tube, cooling to 0° C. for half an hour, heating subsequently to 37° C. for three hours. The presence or absence of hemolysis is observed, and the results compared with those obtained from normal blood treated in the same manner and at the same time.

Third Method.—This technic is carried out *in vitro* in the following manner: Pipet 2 c.c. of the patient's blood in a small test-tube and separate the serum. At the same time place 1 c.c. of blood in a centrifuge tube containing 9 c.c. of a 1 per cent. solution of sodium citrate in normal salt solution. Wash the corpuscles twice and suspend the sediment in 10 c.c. of

¹ Jour. Lab. and Clin. Med., 1921, 6, 218.

² Jour. Amer. Med. Assoc., 1917, lxxii, 1535.

normal salt solution. Then, secure a cubic centimeter of a fresh serum from a normal person. Proceed to make the following mixtures:

Tube 1: 0.2 c.c. patient's serum + 1 c.c. corpuscle suspension.

Tube 2: 0.1 c.c. patient's serum + 1 c.c. corpuscle suspension.

Tube 3: 0.2 c.c. normal serum + 1 c.c. corpuscle suspension.

Tube 4: 0.1 c.c. normal serum + 1 c.c. corpuscle suspension.

Tube 5: 1.0 c.c. corpuscle suspension.

Add sufficient normal salt solution to each tube to make the total volume measure 2 c.c. Shake gently, and place in the refrigerator at a low temperature (not higher than 4° C.) for an hour. Shake each tube gently and place them in the incubator at 37° C. for two hours. The tubes are then centrifuged and the presence or absence of hemolysis is noted. Usually the patient's serum shows hemolysis of greater or less degree.

Similar mixtures may be made with the patient's serum and the corpuscles of a normal person. The hemolytic substance is capable of lysing these to the same degree that it does the patient's own cells.

The Tonicity Test for Measuring the Resistance of Red Blood-corpuscles to Hypotonic Saline Solutions.—The corpuscles are exposed to a series of solutions of varying strengths of sodium chlorid in test-tubes. If the tests are made only occasionally it is better to employ freshly prepared solutions in order to avoid errors due to evaporation of stock solutions.

1. Chemically pure sodium chlorid is dried in a hot-air oven for one hour at about 170° C. A 1 per cent. solution is then prepared by dissolving 5 gm. in 500 c.c. distilled water.

2. A convenient series is 0.7 to 0.2 per cent. solutions of salt with variations of 0.02 per cent. These may be prepared in amounts of 10 c.c. in test-tubes by mixing varying amounts of the stock 1 per cent. solution of salt and distilled water, great care being necessary in pipeting to secure accurate mixtures and results:

0.7 per cent. (7 c.c. of 1 per cent. salt solution + 3 c.c. of distilled water.

0.68 per cent. (6.8 c.c. of 1 per cent. salt solution + 3.2 c.c. of distilled water.

0.66 per cent. (6.6 c.c. of 1 per cent. salt solution + 3.4 c.c. of distilled water.

0.64 per cent. (6.4 c.c. of 1 per cent. salt solution + 3.6 c.c. of distilled water.

0.62 per cent. (6.2 c.c. of 1 per cent. salt solution + 3.8 c.c. of distilled water.

0.6 per cent. (6.0 of 1 per cent. salt solution + 4.0 c.c. of distilled water.

0.58 per cent. (5.8 c.c. of 1 per cent. salt solution + 4.2 c.c. of distilled water.

3. Similar dilutions are made, until the final dilution is reached. In many instances it may not be necessary to use so large a number of dilutions, as from 0.5 to 0.2 per cent. may be sufficient range to indicate the tonicity.

4. Five c.c. of blood are aspirated, under aseptic precautions, from an arm vein of the patient, and immediately placed in 25 c.c. of sterile 1 per cent. sodium citrate in 0.85 per cent. sodium chlorid to prevent coagulation. The flask or large centrifuge tube is gently shaken, and the mixture is centrifuged at sufficient speed to throw down the corpuscles. After this one washing the supernatant fluid is removed, leaving the erythrocytes at the bottom of the tube.

5. A series of small test-tubes (10 by 1 cm.) are marked appropriately and placed in a rack. To each tube 3 c.c. of the various hypotonic salt

solutions and 0.05 c.c. of the red blood-corpuscles (about 1 drop) are added. The salt solution and corpuscles in each tube are well mixed and the whole series is placed in a water-bath for one hour, followed by one or two hours at room temperature, after which the readings are made.

As shown by Lewis¹ the temperature exerts an important influence upon the results, hemolysis becoming progressively more marked as the temperature is decreased from 37° to 5° C. Some observers read one hour after adding the blood and allowing the mixtures to stand at room temperature, the tubes being centrifuged in order to permit accurate readings. Formerly I used a period of eighteen hours in the refrigerator, but have found that the above method permits the settling of the corpuscles to a sufficient degree for accurate readings on the same day as the test is set up and without the extra labor of centrifuging.

6. The tube of lowest dilution—even if it shows but a trace of hemolysis—represents the point of initial hemolysis or *minimal resistance*. The strength of salt solution in which all the corpuscles are hemolyzed represents the point of complete hemolysis or *maximal resistance*.

7. If the test is being frequently used it is good practice to prepare 26 stock solutions of salt varying from 0.7 to 0.2 per cent. in gradations of 0.02 per cent. Used bottles of good grade glass, scrupulously clean and well stoppered to reduce evaporation to a minimum, should be employed.

Short Method.—A short method adapted for occasional use in the clinical laboratory is as follows:

(1) Arrange 11 small test-tubes and carefully pipet the following amounts of a 1 per cent. solution of salt, accurately prepared as described above: 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, and 0.4 c.c.

(2) Add to each tube in order the following amounts of distilled water: 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, and 1.6 c.c. Mix the contents of each tube.

(3) This prepares a series of dilutions of salt as follows: 0.7, 0.65, 0.6, 0.55, etc., to 0.2 per cent. in gradations of 0.05 per cent.

(4) These tubes are carried to the patient. A finger is pricked to secure a *free flow* of blood and 1 drop is added to each tube. The contents are gently mixed.

(5) The readings are made after incubation at 37° C. for one hour followed by two hours at room temperature. Or the tubes may be allowed to stand at room temperature for three hours, when the readings are made. In case of doubt regarding the points of initial and maximal resistance the tubes may be centrifuged.

Method for Measuring the Resistance of Red Blood-corpuscles to Saponin.—(1) A stock 1 : 1000 solution of saponin (Merck's) should be prepared in physiologic saline solution. It is well to prepare this as required by dissolving 0.1 in 100 c.c. saline or 0.01 gm. in 10 c.c.

From this solution twelve dilutions are prepared with saline solution varying from 1 : 25,000 to 1 : 36,000, as follows:

0.1 c.c. of 1 : 1000 + 2.4 c.c. saline = 1 : 25,000.

0.1 c.c. of 1 : 1000 + 2.5 c.c. saline = 1 : 26,000.

0.1 c.c. of 1 : 1000 + 2.6 c.c. saline = 1 : 27,000.

0.1 c.c. of 1 : 1000 + 2.7 c.c. saline = 1 : 28,000, etc.

(2) In a series of test-tubes place 1 c.c. of each solution.

(3) Blood should be collected in citrate solution as described in the tonicity test with hypotonic saline solution. To each tube add 0.1 c.c. of citrated blood, mix, and place in a water-bath for one hour. The readings

¹ Jour. Exper. Med., 1909, 11, 593.

may be made at once with the aid of centrifuging or after the corpuscles have settled for a few hours in a refrigerator.

(4) If washed corpuscles are employed the series of dilutions of saponin should be higher, as, for example, 1 : 35,000 to 1 : 46,000. One drop of washed corpuscle sediment may be employed.

(5) Whole blood may be used, but in this case the dilutions of saponin should be lower. A drop of blood from the finger may be added to each tube. The technic described by Neilson and Wheelon¹ is excellent.

Method for Determining Bacterial Hemolysins.—Two methods may be employed. In the *plate* method 1 c.c. of sterile defibrinated blood is added to 6 to 10 c.c. of 2 per cent. agar, previously boiled and cooled to 40° to 45° C.; the mixture is well shaken, inoculated with the bacteria, and poured into a sterile Petri dish, followed by incubation. Hemolysis is indicated by the presence of clear zones around the colonies.

In the *tube method* the micro-organism is cultivated in an appropriate broth medium and preferably one that is not hemolytic due to protein extractives and salts. Young cultures are to be preferred (twenty-four to forty-eight hours) and may be used unfiltered or the organisms removed by centrifuging. Varying amounts are placed in sterile test-tubes with 1 c.c. of a 1 per cent. suspension of washed rabbit, human, sheep, horse, guinea-pig, or other corpuscles. These mixtures should be incubated in a water-bath at 38° C. for about two hours, when the results may be read at once or after the tubes have rested in a refrigerator over night.

It is well to include a set of controls set up in exactly the same manner, but using a portion of the same broth (sterile) instead of the culture. This will give a check on the hemolytic activity of the culture-medium.

¹ Jour. Lab. and Clin. Med., 1921, 6, 454.

CHAPTER XXI

VENOM HEMOLYSIS

Nature of Venom Hemolysis.—In a previous chapter the statement was made that certain snake poisons, and especially cobra venom, are actively hemolytic. Flexner and Noguchi¹ first demonstrated that the blood-corpuscles of certain species of animals undergo hemolysis when a suitable serum is present, and believed that the venom contained an amboceptor that was active with serum complement.

Shortly afterward Kyes² discovered that venom may hemolyze the corpuscles of certain animals without the presence of serum, and believed that the complement-like activator was contained within the corpuscles, to which he accordingly applied the name endocomplement.

Later Kyes² confirmed Calmette's observation that practically any serum, when heated to 65° C. and higher, showed an increased activity in the process of venom hemolysis. Kyes and Sachs³ then concluded that endocomplement was not of the nature of a thermolabile complement, but was, rather, a combination of lecithin and the stromata of erythrocytes.

Kyes later succeeded in combining cobra venom and lecithin by shaking a watery solution of venom with a solution of lecithin in ether, forming cobra-lecithin, which was found to be actively hemolytic.

The erythrocytes of various animals differ in their susceptibility to venom hemolysis. For instance, those of the dog and guinea-pig are most susceptible to the process; those of the ox, goat, and sheep are entirely refractory, whereas those of the horse, rabbit, rat, pig, and man occupy an intermediate position. Sacks suggested that the variation in hemolytic resistance of red blood-cells from these species of animals was dependent on the amount of lecithin contained in the cells. Kyes, on the other hand, believes that since all erythrocytes contain sufficient lecithin to activate cobra venom, the varying susceptibility depends rather on the availability of the intracellular lecithin for the reaction, *i. e.*, whether the lecithin in the cell is available in a free state.

According to this theory, therefore, any factor that modifies the availability of the cell lecithin may modify the susceptibility of the cells for hemolysis with cobra venom.

Noguchi⁴ has questioned the correctness of this view. He holds that although lecithin exists in the stroma of all kinds of corpuscles, it is not present in a form available for venom activation, and that the degree of susceptibility to hemolysis depends chiefly upon the amount of ether-soluble activators present in the cells, as, for example, fatty acids, particularly oleic acid, and their soluble soaps. In his opinion heating an inactive serum to 65° C. and higher renders it active with venom, owing to the presence of a protein compound of lecithin.

¹ Jour. Exper. Med., 1902, vi, 277.

² Berl. klin. Wchnschr., 1902, xxxix, 886; *ibid.*, 1903, xlii, 21; *ibid.*, 1903, xlii, 956; Biochem. Ztschr., 1907, iv, 109; Jour. Infect. Dis., 1910, vii, 181.

³ Berl. klin. Wchnschr., 1903, xliii, 21, 57, 82.

⁴ Jour. Exp. Med., 1907, ix, 436.

A normal serum may, therefore, contain two activators, one being thermolabile and resembling complement (inactivated by calcium chlorid), and the other being thermostable and a protein lecithin. By adding oleinic acid or its soluble soap to a non-activating serum the latter is rendered highly active so far as venom hemolysis is concerned. Hence while Kyes regards lecithin as the chief component of endocellular complement, Noguchi regards the fatty acids, neutral fats, and soluble soaps as the active agents.

Other observers consider the fatty acids and soap as indirect activating agents in venom hemolysis, in that they possess the power of modifying the cell and rendering the intracellular lecithin available for the formation of complete hemolysin.

On the other hand, in susceptible cells the union of cobra venom and lecithin occurs directly with the formation of the complete hemolysin, Kyes' cobra-lecithid, due to the splitting of the fatty acid radical from the lecithin. Coca,¹ von Dungern and Coca,² and Manwaring³ regard this product as a venom-free lecithin derivative, and not as a lecithin. They prefer to call the active principle "cobralecithinase," and the complete hemolysin "mono-fatty-acid-lecithin."

According to Kyes, the relative amounts of lecithin and venom amboceptor show quantitative relationship comparable to serum amboceptors and complements, namely, that within certain limits, the larger the amount of venom, the smaller the amount of lecithin necessary to effect hemolysis; and, conversely, the larger the amount of lecithin, the smaller the amount of venom required.

Further reference to the intimate relationship that exists between lipoids and complements and hemolysis is also made in the discussion on the nature of complements.

VENOM HEMOLYSIS IN SYPHILIS

The first application of venom hemolysis was made by Weil,⁴ who found, in testing the hemolytic powers of cobra venom with cells derived from persons suffering from different diseases, that the red cells of syphilitic individuals offered a characteristic resistance. Various explanations have been offered for this phenomenon:

1. It was argued that the quantity of red-cell lecithin is actually diminished in syphilis after the primary stage because pallidum toxin attacks the same lipoidal substances of tissue cells as does cobra venom, in this way accounting for the diminished amount of lecithin that can be extracted in syphilis, as compared with that obtained from normal tissues. Accordingly, the increase in resistance is only apparent, and is due rather to the fact that there is insufficient endocomplement for the venom amboceptor.

2. Another explanation offered was that the increased resistance of the red cells of syphilitic persons to venom hemolysis is due to the fact that pallidum toxin attacks endocomplement, and that the cells become specifically immunized to this deleterious influence in much the same way that repeated injections of such a hemolytic agent as saponin leads in rabbits to the production of red cells, which show a marked resistance to saponin hemolysis, but not to any other hemolytic agent.

3. Pallidum toxin was believed to so affect the lecithin content of red

¹ Ztschr. f. Immunitätsf., 1912, 12, 134.

² Jour. Infect. Dis., 1912, 10, 57.

³ Ztschr. f. Immunitätsf., 1910, 6, 513.

⁴ Proc. Soc. Exper. Biol. and Med., 1909, vi, 49; *ibid.*, 1909, vii, 2; Jour. Infect. Dis., 1909, vi, 688.

cells as to render a smaller quantity of it available in a free state for union with the venom amboceptor to form the hemolysin.

4. Another theory advanced was that pallidum toxin effects a dissociation of red cells between lecithin and cholesterol, the latter substance causing inhibition of hemolysis.

Whatever may be the true explanation, the fact has been quite well attested that the red cells of a large percentage of persons in the tertiary stage of syphilis exhibit a characteristic increased resistance to venom hemolysis, and while the cobra hemolysis test in this disease is of secondary importance to the Wassermann reaction as a diagnostic procedure, yet it represents one of the most interesting of biologic phenomena, and may possibly be employed in other clinical methods.

TECHNIC OF THE COBRA VENOM TESTS

Preparation of Venom Solution.—A 1 : 1000 stock solution of dried cobra venom is prepared by accurately weighing out 0.01 gm. of dried pulverized venom and dissolving this in 10 c.c. of normal saline solution (1 : 1000). This stock dilution is best preserved in amounts of 1 c.c. in sealed ampules, kept in the frozen state in the ice chest in a wide-mouthed well-stoppered vacuum bottle containing salt and ice (Schwartz).

Each cubic centimeter is sufficient for making three tests, so that the 10 ampules will be enough for 30 tests, or 1 gm. of venom for 3000 reactions. Or the dried venom may be weighed out in amounts of 0.001 gm. in test-tubes, and diluted, just before being used, with 1 c.c. of normal salt solution (1 : 1000). Immediately before the tests are conducted subdivisions are prepared of the stock dilution (1 : 1000), using separate pipets for each, as follows:

Solution A: 1 : 10,000 = 1 c.c. stock solution + 9 c.c. normal saline solution.

Solution B: 1 : 15,000 = 2 c.c. solution A + 1 c.c. normal saline solution.

Solution C: 1 : 20,000 = 1 c.c. solution A + 1 c.c. normal saline solution.

Solution D: 1 : 30,000 = 1 c.c. solution B + 1 c.c. normal saline solution.

Solution E: 1 : 40,000 = 1 c.c. solution C + 1 c.c. normal saline solution.

These amounts are sufficient for making three tests; if more tests are to be made, larger amounts of the various dilutions will keep fairly well in a good refrigerator for several days, but it is always well to plan the work so that the exact amount will be prepared and no waste occur.

Each lot of stock solution should be tested occasionally with the cells of known normal and positive persons, to make certain that the venom is active in these dilutions. These titrations are conducted in the same manner as the test.

Preparation of Blood-cells.—With a sterile syringe blood is drawn from a vein at the elbow and 2 c.c. placed in an accurately graduated centrifuge tube containing 5 c.c. of a 2 per cent. solution of sodium citrate in normal saline solution. The suspension should be shaken gently to insure mixing and the prevention of coagulation, but *defibrination by means of whipping should never be practised*. The cells may be prepared at once or placed in the ice-chest overnight. Sufficient normal saline solution is added to bring the total volume to 15 c.c. Mix gently and centrifuge at *low speed* until

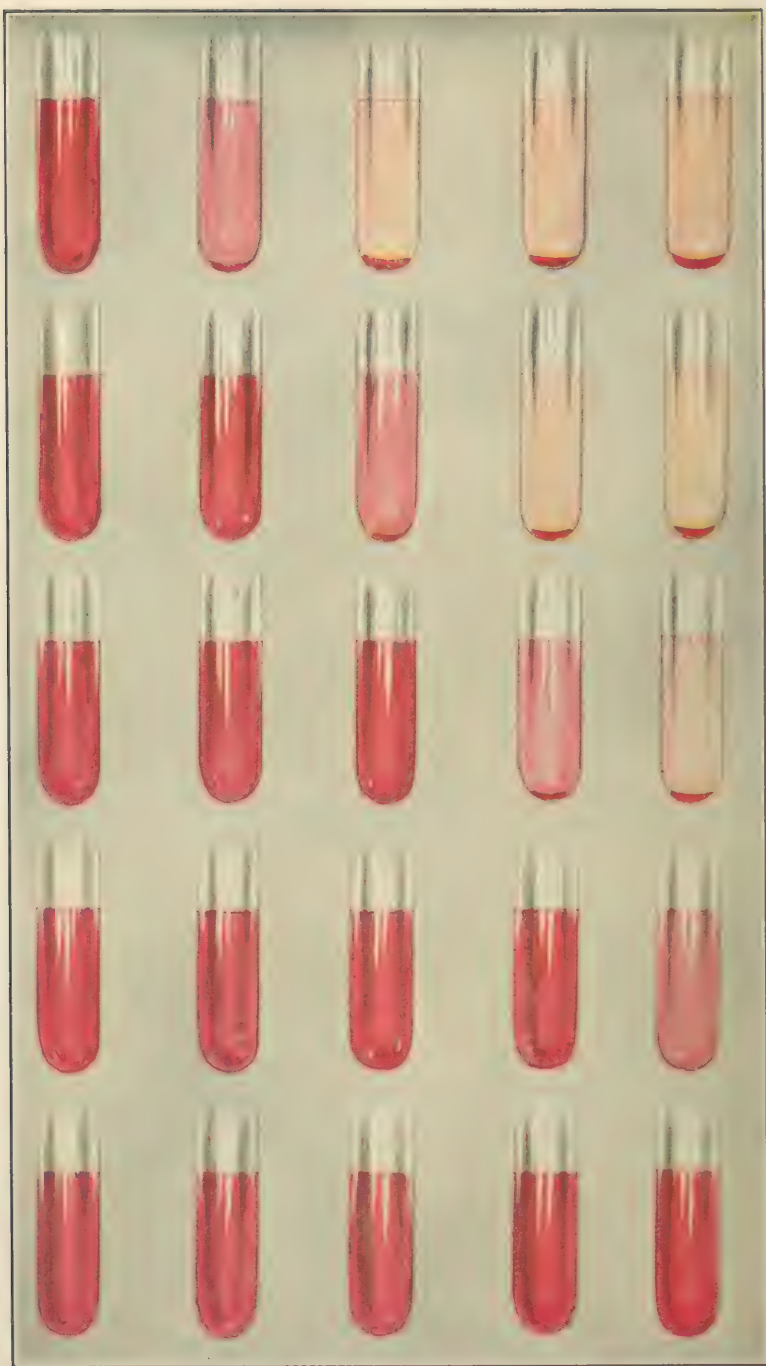


FIG. 128.—VENOM HEMOLYSIS.

First row strongly positive; second row moderately positive; third row weakly positive; fourth and fifth rows negative.

the supernatant fluid is clear. Draw off the fluid, add more normal saline solution, mix up the cells, and centrifuge again until clear. Repeat this process once more so that all traces of serum will be removed. After completing the last centrifugalization, which should be thorough (ten minutes), the cells are diluted with 25 volumes of normal saline solution, which makes a 4 per cent. suspension—for instance, 0.8 c.c. of corpuscles would require 20 c.c. of diluent.

Just what influence sodium citrate has upon the process is not known, but it is certain that satisfactory results are seldom obtained with blood defibrinated by whipping with rods of glass beads. Similarly, if centrifuged too rapidly, cells are broken up or rendered more susceptible to the venom amoceptor.

The Test.—Into a series of 5 test-tubes (4 by $\frac{1}{2}$ inches) place 1 c.c. of each dilution of venom, and label each tube correctly; add 1 c.c. of the 4 per cent. suspension of cells to each tube, shake gently, and incubate for one hour at 37° C. Except in cases where the venom dilutions are being frequently used and are known to be reliable, controls should be included. I usually add a normal control of cells from a healthy person with the 1 : 30,000 or 1 : 40,000 dilution and expect complete hemolysis to occur. A preliminary reading is made at the end of an hour; the tubes are shaken gently and placed in the refrigerator overnight; the final reading is made next morning, and should tally quite closely with the preliminary reading.

Reading the Results.—Unless the cells are derived from a very strongly reacting case of syphilis, the 1 : 10,000 dilution will be hemolyzed. The normal control tube should show complete hemolysis. If the 1 : 10,000 tube is not hemolyzed and some of the higher dilutions show hemolysis, an error in technic has occurred, and the test should be repeated with fresh dilutions.

The reactions are read and recorded as follows (Fig. 128):

A	B	C	D	E	
H.	M. H.	—	—	—	= strongly positive.
H.	H.	M. H.	—	—	= moderately positive.
H.	H.	H.	S. H.	—	= weakly positive.
H.	H.	H.	H.	M. H.	= negative.
H.	H.	H.	H.	H.	= certainly negative.

H. = complete hemolysis; M. H. = marked hemolysis; S. H. = slight hemolysis; the dash (—) = no hemolysis.

If complete hemolysis has occurred in all tubes after an hour's incubation, the cells are regarded as being hypersensitive to cobra venom. This occurs, as a rule, in primary syphilis and in active tuberculosis.

PRACTICAL VALUE OF THE VENOM TEST IN SYPHILIS

1. While the test is much simpler than the Wassermann reaction and there is less possibility for errors in technic to creep in, it possesses but two other advantages, namely: (1) It may react positively in latent or tertiary syphilis when the Wassermann reaction may be negative, and (2) it may react positively in treated syphilitic cases when the Wassermann reaction is negative, and thus point to a continuation of treatment. Corson-White and Ludlum¹ found 94 per cent., Schwartz² 69.3 per cent., and Stone

¹ Jour. Nervous and Mental Diseases, 1910, xxxvii, 721.

² New York Medical Journal, 1912, xcv, 23.

and Schottstaedt¹ 90.9 per cent. of positive reactions in the active stages of syphilis.

2. The test is positive in but about 20 per cent. of cases of *tabes dorsalis* and general paralysis (White and Ludlum), a finding obviously inferior to the Wassermann reaction.

3. During primary syphilis the cells are hypersensitive and positive reactions are but occasionally obtained.

4. Positive reactions may occur in cancer, but otherwise the test is quite specific, and may, in selected cases, prove a valuable adjunct to the Wassermann reaction. However, with a more improved technic in performing the Wassermann reaction, and especially if antigens reinforced with cholesterin are used, the venom test is inferior to the Wassermann. In cases where syphilis or tuberculosis of the lungs is to be differentiated, a negative venom test would indicate tuberculosis, as in this disease the cells are hypersensitive.

THE PSYCHOREACTION OF MUCH AND HOLZMANN

Normal serum, when added to a lytic dose of cobra venom and human red blood-cells, will not interfere with hemolysis. According to Much and Holzman,² however, if the serum obtained from a patient suffering from depressive mania or dementia præcox is added to the mixture of venom and human red blood-cells, the expected hemolysis does not take place.

Technic.—A 1 : 5000 dilution of *cobra venom* is prepared by diluting 1 c.c. of the stock dilution (p. 416) with 4 c.c. of normal saline solution. Enough of the patient's blood is collected from a vein at the elbow to yield at least 1.5 c.c. of serum; heat the serum to 55° C. for an hour. Prepare a 5 per cent. suspension of *washed human blood-cells*. An effort should be made, if possible, to use as a control the cells of a person which are known to be readily hemolyzed by 1 c.c. of the 1 : 5000 dilution of venom in half an hour.

Into a series of three small test-tubes place 0.4 c.c. of the patient's serum and decreasing amounts of venom—1, 0.8, and 0.5 c.c. respectively. Next add to each tube 1 c.c. of the blood-corpuscule suspension. The total volume in each tube is brought up to 2.5 c.c. by the addition of normal saline solution.

A similar series of tubes should be set up as *controls*, the patient's serum being omitted. The following table shows the arrangement:

Tube 1: 0.4 c.c. serum + 1 c.c. venom + 1 c.c. blood.

Tube 2: 0.4 c.c. serum + 0.8 c.c. venom + 1 c.c. blood.

Tube 3: 0.4 c.c. serum + 0.5 c.c. venom + 1 c.c. blood.

Tube 4: 1 c.c. venom + 1 c.c. blood.

Tube 5: 0.8 c.c. venom + 1 c.c. blood.

Tube 6: 0.5 c.c. venom + 1 c.c. blood.

Tube 7: 0 1 c.c. blood.

The tubes are shaken gently and incubated for an hour at 37° C., when a preliminary reading is made. If the control tubes 4, 5, and 6 are hemolyzed, a positive reaction would be indicated by the inhibition of hemolysis in the first three tubes, 1, 2, and 3. Control tube 4 at least should be completely hemolyzed at the end of half an hour, and in a positive reaction tube 1, containing the same amount of venom with the patient's serum, will show inhibition of hemolysis.

¹ Arch. of Int. Med., 1912, x, 8.

² Münch. med. Wchn., 1909, 20.

Practical Value.—Corson-White and Ludlum¹ have found the reaction positive in 80 per cent. of cases of the catatonic form of dementia præcox and in 62 per cent. of the hebephrenic type. Only 3 out of 37 cases of manic-depressive insanity reacted positively. Among controls with serums of other diseases positive reactions were secured in one case each of cerebro-spinal syphilis, tertiary lues, exophthalmic goiter, and confusional insanity, and in 2 cases each of general paralysis and epilepsy. The aforementioned observers claim, however, that if the venom is carefully standardized with blood-cells that are completely hemolyzed in 1 : 5000 dilution of venom in thirty minutes, the reaction possesses some diagnostic value, having been found, under these conditions, to yield positive reactions in 87 per cent. of cases of dementia præcox and in 100 per cent. of catatonics.

In a study of this reaction Rosanoff² found that practically all human sera possess the power, in a more or less pronounced degree, of inhibiting the hemolytic action of cobra venom on human corpuscles. A high degree of inhibition of hemolysis by serum was found among 8.1 per cent. of apparently healthy persons and was not specific for any psychosis, although the reaction was present in 57.9 per cent. of a series of cases of dementia præcox. No relationship was found between the Wassermann and Much-Holzmann reactions.

Moss³ has likewise reported unfavorably upon the diagnostic value of this reaction. The action of different sera in favoring or retarding venom hemolysis was inconsistent.

According to Citron, the reaction is probably due to interference with hemolysis by an increase in the cholesterin of the serum—a possibility more apt to occur in diseases of the central nervous system than in any physiologic or other pathologic condition.

VENOM HEMOLYSIS IN TUBERCULOSIS

Calmette found that the blood of tuberculous patients may activate cobra hemolysin in very small doses, and upon this observation he devised a test that yielded about 65 per cent. of positive reactions in tuberculosis. Positive reactions have, however, been found in other diseases, and the practical value of the test has not been established.

VENOM HEMOLYSIS IN CANCER

Although the red blood-corpuscles of the horse may be hemolyzed by venom without the aid of serum, Kraus, Graff, and Ranzi^{4, 5} found that about 70 per cent. of cancer serums considerably hastened and aided the hemolytic process.

A 1 : 5000 dilution of venom is used. In two series of 4 tubes each place respectively 0.1, 0.2, 0.3, and 0.5 c.c. of the patient's serum (heated); to the first series add 0.3 c.c. of the venom solution, and to the second series 0.15 c.c. of the same. To each of the tubes in the series add 5 drops of a 10 per cent. suspension of washed horse corpuscles; shake thoroughly and incubate at 37° C. Inspect the tubes at the end of fifteen and thirty minutes, and then after one, two, and three hours.

Positive reactions have also been found in pregnancy after the fourth month, in icterus, advanced tuberculosis, and other diseases.

¹ Jour. Nerv. and Mental Diseases, 1910, xxxvii, 721.

² Arch. Int. Med., 1909, 4, 405.

³ Bull. Johns Hopkins Hosp., 1911, 22, 278.

⁴ Wien. klin. Wchn., 1911, No. 28.

⁵ Münch. med. Wchn., 1912, No. 59, 574.

CHAPTER XXII

PRINCIPLES OF THE BORDET-GENGOU PHENOMENON OF COMPLEMENT FIXATION

Historic.—With Bordet's discovery of the hemolysins in 1898, and his demonstration of the rôle of the antibody or sensitizer and alexin in the process, new light was thrown upon the bacteriolysins, and the close analogy between hemolysis and bacteriolysis soon became apparent. Bordet's discoveries were quickly verified by Ehrlich and Morgenroth and the German school in general, although his views regarding the mechanism of the processes were questioned. The controversy soon centered upon the question of the unity or the multiplicity of alexins or complements. Bordet at this time advanced his belief in the existence of one alexin or complement that would act with any sensitizer or amboceptor, and he still maintains this view. One of the experiments conducted by him, and later made in conjunction with his pupil, Gengou, in support of his theory, is now known as the *Bordet-Gengou phenomenon of complement fixation*.¹ This has become widely known as the precursor of all complement-fixation tests, and is the basis of the well-known and invaluable Wassermann reaction for the diagnosis of syphilis.

In devising the technic of this important method Bordet's main object was to show that the complement in a normal serum would unite with either a bacteriolytic or a hemolytic amboceptor, and that, by furnishing sufficient of either amboceptor, all the complement may be "fixed." He argued that if two or more complements existed in the same serum, as was held by Ehrlich, they would demonstrate their presence by exhibiting different affinities for these widely varying amboceptors.

Prior to this Bordet had shown that the addition of a small amount of normal serum to an immune hemolysin would result in lysis of the homologous corpuscles, and that the process could not take place without the alexin. He then mixed an emulsion of pest bacilli with antipest serum and added a small amount of normal, unheated guinea-pig serum to supply the alexin or complement. After allowing the mixture to stand for four hours at room temperature, it was sought to determine whether the alexin had been fixed by pest antigen and pest amboceptor, or whether it was free in the fluid. Bordet knew that the normal alexin serum used in the experiment could produce hemolysis of corpuscles with a homologous amboceptor, so he tested for free alexin by subsequently adding to the mixture antirabbit hemolysin and rabbit corpuscles. Hemolysis did not occur because the alexin or complement had been bound by the pest antigen and amboceptor. When a normal serum was substituted for the antipest serum, hemolysis occurred, because the normal serum contained no sensitizers or amboceptors that could unite with the pest bacilli and "fix" the alexin, which, therefore, remained free, and when the hemolysin and red corpuscles were subsequently added, united with them to lyse the red cells. In this way the corpuscles and hemolysin served as indicators for free or unfixed alexin or complement, just as litmus or phenolphthalein may be used as a test for the presence of an acid or an alkali.

¹ Ann. d. l'Inst. Pasteur, 1901, 15, 289.

The Original Complement-fixation Reaction.—In this paper Bordet and Gengou¹ gave the results attained with three different antigens and their respective antisera—pest, typhoid, and *Proteus vulgaris*. The details of the technic are given with an antigen of pest bacilli and an antipest horse-serum are given:

(a) The antigen consisted of twenty-four-hour cultures of pest bacilli in normal salt solution, making a somewhat concentrated emulsion.

(b) The antipest horse-serum was heated for half an hour at 56° C., to remove the alexin or complement. Normal horse-serum (heated) was also employed as a negative control.

(c) As alexin, the fresh serum of a normal guinea-pig was used.

(d) The substance sensibilisatrice or hemolysin consisted of the inactivated serum of a guinea-pig injected with rabbit's red blood-cells.

(e) The corpuscles of the rabbit were washed, to free them of alexin, and were used as the indicator antigen.

To 0.4 c.c. of the pest emulsion, 1.2 c.c. of inactivated antipest serum and 0.2 c.c. of guinea-pig alexin were added. This mixture was allowed to remain at the ordinary laboratory temperature (18°–20° C.) for several hours. In order to ascertain whether or not the alexin had been absorbed, hemolysin and erythrocytes were added to the mixture. This was accomplished by sensitizing about 20 drops of washed rabbit's cells with 2 c.c. of inactivated hemolysin for about fifteen minutes, and adding 2 drops to each of the test-tubes. Hemolysis did not occur because the alexin had been fixed by the pest antigen and antibody. A similar test, conducted with normal serum, hemolyzed in a few minutes because the complement or alexin remained free in the mixture. Even at this early stage Bordet included the important controls on his antigen and serums that are so necessary in all complement-fixation tests.

The following table gives the original details and results of the first complement-fixation experiment with pest antigens and antipest serum:

THE ORIGINAL BORDET-GENGOU COMPLEMENT-FIXATION REACTION

TUBE.	ANTIGEN, C.C.	SERUM.	ALEXIN, C.C.	HEMOLYSIN AND ERYTHRO- CYTES.	RESULTS.
(a)...	0.4	1.2 c.c. inactivated anti- pest serum.	0.2	2 drops sensitized rab- bit's blood.	No hemol- ysis.
(b)....	0.4	1.2 c.c. inactivated normal serum.	0.2	2 drops sensitized rab- bit's blood.	Complete hemolysis.
(c)....	...	1.2 c.c. inactivated anti- pest serum.	0.2	2 drops sensitized rab- bit's blood.	Complete hemolysis.
(d)....	...	1.2 c.c. inactivated normal serum.	0.2	2 drops sensitized rab- bit's blood.	Complete hemolysis.
(e)....	0.4	1.2 c.c. inactivated anti- pest serum.	...	2 drops sensitized rab- bit's blood.	No hemol- ysis.
(f)....	0.4	1.2 c.c. inactivated normal serum.	...	2 drops sensitized rab- bit's blood.	No hemol- ysis.

By showing in this manner that the complement of a serum could be fixed by either bacteriolytic or hemolytic amboceptors, Bordet endeavored to support his views on the unity of complement. Ehrlich and Morgenroth later verified his findings, and, in addition, by more delicate and complicated experiments, showed that many complements may be present

¹ Ann. d. l'Inst. Pasteur, 1901, xv, 289.

in a serum, a fact manifested by a different rate of absorption, by the action of specific anticomplements, etc., as mentioned in a previous chapter.

While Ehrlich's theory as to the multiplicity of complements has been widely accepted, the subject really possesses greater academic than practical interest, for experience has shown that the results are the same in complement-fixation tests at least, regardless of whether we believe in the unity or in the multiplicity of complement, as under ordinary conditions the complement or complements in a given quantity of serum are capable of being absorbed by bacteriolytic, hemolytic, or other amboceptors.

History of Applications of the Bordet-Gengou Phenomenon; Discovery of the Wassermann Reaction.—Gengou¹ showed later that not only cellular antigens, such as bacteria and red blood-cells, are capable of stimulating the production of amboceptors, but that the proteins in solution, such as serum and milk, may produce complement-binding amboceptors in addition to precipitins. This subject was later studied more extensively by Moreschi,² whose interest became aroused as the result of his theoretic studies upon anticomplements. This investigator observed that upon mixing a soluble protein with its antiserum precipitation occurred and the existing complement disappeared, a coincidence that led him to assert that the complement disappeared because it was carried down mechanically in the precipitate. This explanation naturally had the effect of leading many to assume that the Bordet-Gengou phenomenon of complement fixation may be the result of a similar precipitation process, and led to many interesting and valuable investigations, especially those made by Gay.³ It is now generally agreed, however, that protein amboceptors are formed, and that actual complement fixation occurs independently of precipitation. Later Neisser and Sachs⁴ elaborated on Gengou's studies, and perfected a complement-fixation technic for the differentiation of proteins that is much more delicate than the precipitin test, and serves to demonstrate and differentiate traces of protein, as in blood-stains, so minute in quantity as not to be appreciable by the precipitin test.

Widal and Lesourd⁵ applied the Bordet-Gengou reaction to the diagnosis of typhoid fever, using an emulsion of typhoid bacilli and the serum of a typhoid fever patient, and found that a positive reaction occurred more frequently and earlier than the agglutination test. These observations were made soon after Bordet and Gengou's discovery, and were probably the first direct and practical application of a complement-fixation technic in diagnosis. It was not until several years later, however, that the possibilities of the method were seriously considered.

Hitherto most experiments were conducted with known antigens and their antibodies. It was shown, especially in the work of Neisser and Sachs on protein differentiation, that when an antigen and its specific antibody are present complement is absorbed, and the specific relation existing between these bodies was again emphasized. *Hence in a complement-fixation test, if the antibody is known the antigen may be found, or if the antigen is known the antibody may be found, the detection in either instance depending upon whether or not complement is absorbed, this being decided by adding corpuscles and their amboceptors to the mixture, the absence or the occurrence of hemolysis determining this point.* In this manner Neisser and Sachs were able to diagnose the nature of blood-stains by using solutions of the suspected stains as antigen, and adding, in different experiments, known anti-

¹ Ann. d. l'Inst. Pasteur, 1902, 16, 734.

² Berl. klin. Wchn., 1905, No. 37, 1181.

³ Centralbl. f. Bakteriöl., 1905, 39, 603.

⁴ Berl. klin. Wchn., 1905, xlii, 1388.

⁵ Comp. rend. Soc. biol., 1901, 53, 841.

serums secured by injecting rabbits with various bloods. When a positive complement-fixation reaction occurred, they concluded that the antigen of the blood corresponded to the known antibody, and they were thus able to identify the species of animal from which the blood in the stain was derived.

Wassermann and Sachs, encouraged by these results, endeavored by complement-fixation tests to show the existence of antigens in diseased organs, using tuberculous glands and lungs with an antituberculous serum and the serums of tuberculous persons. These investigations finally led to the serum diagnosis of syphilis, in which the antigen was supplied by tissues containing large numbers of the *Spirocheta pallida*. By furnishing the antigen it was hoped that the luetic antibody could be detected in the body fluids through the absorption of complement by the union of the antigen and its antibody in the complement-fixation test.

At this time Schaudinn and Hofmann discovered the spirochete of syphilis, a finding that served to focus the attention of the medical world upon this disease. In co-operation with Neisser, who was conducting extensive researches on experimental syphilis in monkeys, Wassermann and Bruck¹ applied the complement-fixation method to the study of these experimental infections, and published a report of their work on May 10, 1906.

At first monkeys were immunized with aqueous extracts of human chancre, condylomata, syphilitic placenta, etc., and their serums, mixed *in vitro* with these extracts, were found to give the complement-fixation reaction. Since these results may have been due to protein amboceptors or precipitins produced simultaneously by the injection of human serum contained in the extracts, the experiment was carried out with extracts of bone-marrow and other organs of syphilitic monkeys used to obviate this error. It was found, however, that the inactivated serums of syphilitic monkeys reacted positively with antigens of either human or monkey lesions, and regardless of whether the monkeys had been injected with human extracts, since, after ordinary cutaneous infection, their serum would show complement fixation. These early reports also showed a high specificity for complement fixation, as monkey immune serum did not react with extracts of normal organs or normal monkey serum with extracts of syphilitic organs.

Just fourteen days after Wassermann, Neisser, and Bruck published their report, a second paper on the same subject appeared, showing the work of Detre.² Using aqueous extracts of luetic papules, liver, pancreas, and tonsillar exudate as antigens Detre performed the complement-fixation method with the serums of 6 syphilitic and 4 normal persons, finding positive reactions with two of the six luetic serums.

Early Work on the Wassermann Reaction; Discovery of Its Non-specific Character.—In 1906 Wassermann and Plaut³ studied the cerebrospinal fluids of 41 cases of paresis, and found positive reactions in 32, 4 cases reacting doubtfully and 5 negatively. In the following year Levaditi and Marie⁴ and Schutze⁵ observed positive reactions with the cerebrospinal fluid of tabetics, whereas Morgenroth and Stertz⁶ confirmed the previous finding in paresis. Since then numerous investigators have corroborated these observations, and while all the evidence tended to strengthen the belief

¹ Deutsch. med. Wchn., 1906, 32, 745.

² Wien. klin. Wchn., 1906, 19, 619.

³ Deutsch. med. Wchn., 1906, 32, 1769.

⁴ C. R. Soc. Biol., 1907; Sem. med., 1907, No. 21.

⁵ Allg. Ztschr. f. Psych. u. Psychiat.-gerichtl. Med., 1908, lxxv, 565.

⁶ Virchow's Archiv., 1907, clxxvii, 166.

in the luetic origin of general paralysis and tabes, decisive confirmation was lacking until Noguchi and Moore,¹ in 1913, demonstrated the presence of the *Treponema pallidum* in sections of the cerebral cortex.

In 1906 Wassermann, Neisser, Bruck, and Schucht² applied the complement-fixation test to a large number of cases of syphilis in Neisser's clinic. Aqueous extracts of luetic liver, placenta, glands, chancres, and gummata were used as antigens. Of 257 cases in all stages of the disease, only 49 reacted positively. With but 19 per cent. positive reactions, the method did not appear to have a promising future, although at the present time, with a better understanding of the technic and of the importance of quantitative factors that greatly influence the results, the value of the test has been greatly enhanced.

As it appeared that after all no method of diagnosis was to be secured as the result of the demonstration of the syphilitic antibody in the body fluids, Neisser and Bruck determined to return to earlier methods and attempt to discover if luetic antigen could be demonstrated in the serums of luetics through complement fixation. Antigens prepared of the red corpuscles of syphilitic persons gave positive reactions with the serums of highly immunized monkeys. Of 160 luetic patients, in 70 per cent. either antigen or antibody was found. Later, however, Citron showed that the extracts of corpuscles of normal persons yielded similar results which, in the light of subsequent discoveries, was due to their content in lipoidal substances.

Up to this time the syphilitic reaction was considered as but a simple and direct application of Bordet's phenomenon, requiring a specific syphilitic antigen before complement could be fixed with the syphilis antibody. In January, 1907, Weygandt³ reported that he had obtained a positive reaction in tabes with an extract of normal spleen. Marie and Levaditi, using an aqueous extract of normal fetal liver, secured positive reactions with the cerebrospinal fluid of paretics, but observed that it was necessary to use larger doses than when extracts of syphilitic organs were used. Subsequently other investigators, as, for example, Fleischmann,⁴ Michaëlis,⁵ Landsteiner, and Plaut, found that watery extracts of normal organs served to fix the complement with luetic antibody. Finally, in December, 1907, a profound impression was created by the discovery made by Landsteiner, Müller, and Pötzl⁶ that an alcoholic extract of guinea-pig heart yielded results equal to those obtained with an aqueous extract of syphilitic liver. These results indicated that the antigenic principle was soluble in alcohol, and a prolonged series of investigations on the various lipoids and their relation to the reaction was begun. These included the employment of lecithin by Porges and Meier⁷; sodium taurocholate and glycocholate by Levaditi and Yamonouchi⁸; cholesterin and vaselin by Fleischmann; oleic acid by Sachs and Altman⁹; acetone-insoluble fractions of alcoholic extracts by Noguchi and Bronfenbrenner,¹⁰ and many other combinations of various lipoidal substances by different investigators.

These dealt a blow to the theory of the Wassermann reaction, which, taken in conjunction with the wide-spread use of the test by inexperienced and unskilful persons and the many sources of error, tended to retard an earlier appreciation of the great value of the test, and served to swing the

¹ Jour. Exper. Med., 1913, 17, 232.

² Ztschr. f. Hyg., 1906, lv, 451.

³ Münch. med. Wchn., 1907, liv, 1557.

⁴ Dermat. Centralbl., 1908-09, 11, 226, 258.

⁵ Berl. klin. Wchn., 1907, xlv, 1103.

⁶ Wien. klin. Wchn., 1907, 20, 1421, 1565.

⁷ Berl. klin. Wchn., 1908, xlv, 731.

⁸ C. R. Soc. Biol., 1908, lxiv, 814.

⁹ Berl. klin. Wchn., 1908, xlv, 494.

¹⁰ Jour. Exper. Med., 1911, 13, 43.

pendulum of medical opinion so far in the wrong direction that it is only now, with a better understanding of its possibilities and limitations, that the method is being established in its proper sphere. Positive reactions were said to have occurred in frambesia, leprosy, malaria, pellagra, pneumonia, scarlet fever, typhoid fever, malignant tumors, and practically every other disease liable to afflict humanity. The careful work of Citron¹ was largely instrumental in preserving the importance of the reaction until a better understanding of the technic resulted in improved and more careful work, with a greater respect for the real value of the Wassermann reaction.

Although the true explanation of the mechanism of complement fixation in syphilis is still lacking, sufficient work has been done to show that the specific nature of the reaction is dependent upon a peculiar luetic antibody, and that the older belief in the specificity of antigen, in so far as it insisted upon the presence of the *Treponema pallidum* in the tissues extracted for "antigen," is largely disproved. While the method cannot be said to be absolutely diagnostic of syphilis, since positive reactions were had in frambesia (yaws), yet it is practically so, especially in those countries where the latter disease is unknown or relatively infrequent.

Kinds of Antigens Inducing the Production of Complement-fixing Antibodies.—From this brief historic survey it is apparent that many different substances are capable of acting as antigens in inducing the production of complement-fixing antibodies (for which I propose the name *alexofixagens*). Practically every foreign protein is capable of antigenic stimulation, although substances belonging to the same class may vary greatly in antigenic activity; for example, among the bacteria, the bacilli of the typhoid-colon group are actively antigenic, while the pneumococcus is quite feeble. *Treponema pallidum* (syphilis) and *T. pertenu* (frambesia tropica) are the most active antigens. The substances known to be antigenic in inducing complement-fixing antibodies may be classified as follows:

- | | | |
|--|--|--|
| 1. Foreign cells | 1. Bacteria, fungi, and protozoa | <div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <p><i>T. pallidum</i>
<i>T. pertenu</i>
<i>B. mallei</i>
<i>B. abortus</i> (Bang)
<i>B. typhosus</i>
<i>B. diphtheriae</i>
<i>Gonococcus</i>
<i>Sporotrichia</i>
<i>Helminths</i></p> </div> <div style="font-size: 3em; margin-right: 10px;">}</div> <div>Active</div> </div> |
| | | <div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <p><i>B. tuberculosis</i>
<i>Pneumococcus</i>
<i>Streptococcus</i>
<i>Staphylococcus</i>
<i>Spore-bearing bacilli</i></p> </div> <div style="font-size: 3em; margin-right: 10px;">}</div> <div>Feeble</div> </div> |
| 2. Erythrocytes, leukocytes, spermatozoa, and other cells. | | |
| 2. Foreign soluble substances | 1. Sera. | |
| | 2. Albuminous exudates and transudates. | |
| | 3. Milk and other albuminous excretions. | |
| | 4. Plant proteins. | |

In other words, *all substances known to induce the production of cytolytins, albuminolytins, and precipitins* during infection or by artificial immuniza-

¹ Deutsch. med. Wchn., 1907, 33, 1165; Berl. klin. Wchn., 1907, xlv, 1370; 1908, xlv, 518.

tion may act as *alexofixagens*, that is, induce the production of alexin or complement-fixing antibodies; some substances, however, may not induce the production of demonstrable amounts of precipitins and yet act as alexofixagens.

Chemical Nature of Alexofixagens.—As discussed in previous chapters antigens are regarded as protein molecules, and those producing complement-fixing antibodies are apparently of the same nature.

The physical and chemical state of the protein, however, bears an important relation to its activity in inducing the production of these antibodies. Solubility in the tissues appears to be an essential character and proteins that have been boiled (coagulated) appear to lose their antigenic activity.

Purified vegetable and animal proteins may act as antigens, as shown by White and Avery for edestin,¹ Lake, Osborne, and Wells² for wheat and rye gliadins and hordein of barley, and Kahn and McNeil³ for edestin, phaseolin, casein, and lactalbumin. Incomplete proteins appear to be devoid of antigenic activity. Gelatin which lacks cystine, tyrosin, and tryptophan was found by Starin,⁴ Kahn and McNeil without antigenic activity, and the same was found true of the protamins and histones by Wells,⁵ Schmidt,⁶ and others.

The cleavage products of proteins have not been studied in relation to the production of complement-fixing antibodies as thoroughly as in relation to precipitin production and anaphylaxis. Schmidt⁷ has found deuto-albumose obtained from Witte's peptone without antigenic activity. Gay and Robertson⁸ found the split products of casein inactive; Kahn and McNeil⁹ and Fink¹⁰ have reported similar results with proteoses. The amino-acids and simple polypeptids are not antigenic. These investigations have indicated, therefore, that the antigenic activity of proteins depends upon their chemical structure, and if split or modified by racemization they lose in whole or part their antigenic activity.

Some investigators have declared that the "nucleoproteins" are actively antigenic and highly specific. So-called "nucleoproteins" have been prepared from bacteria and tissue cells, but, as pointed out by Wells,¹¹ a large part of the reported results of experiments with these is to be disregarded because of the indefinite chemical nature of the substances employed. According to Wells, the nucleoproteins concern three substances from the immunologic standpoint: (1) Nucleic acid, which is non-protein, probably a glucosid and non-antigenic; (2) the nucleins, which seem to consist of nucleic acid firmly bound to proteins and especially the histones and perhaps to protamins. The histons, protamins, and nucleic acid are not antigenic, and the nucleins composed of these radicals probably are not. (3) The nucleoproteins, which appear to be indefinite and loose compounds of any or all of the proteins of the cell with either nucleic acid or with the nucleins. These are antigenic to the degree depending upon the amount of protein present, but these are not an essential part of nucleoprotein and the amount present depends largely upon the method employed. In other words, pure nucleoproteins from the chemical standpoint do not appear to be antigenic; so-called "nucleoproteins" loosely prepared are antigenic to the degree depending upon the amount of protein present.

¹ Jour. Infect. Dis., 1913, 13, 103.

² Jour. Infect. Dis., 1914, 14, 364.

³ Jour. Immunology, 1918, 3, 277.

⁷ Univ. of Californ. Pub. in Path., 1916, 2, 157.

⁸ Jour. Exper. Med., 1912, 16, 470.

⁹ Jour. Immunology, 1918, 3, 277.

⁴ Jour. Infect. Dis., 1918, 23, 139.

⁵ Ztschr. f. Immunitätsf., 1913, 19, 599.

⁶ Jour. Biol. Chem., 1920, 41.

¹⁰ Jour. Infect. Dis., 1919, 25, 97.

¹¹ Ztschr. f. Immunitätsf., 1913, 19, 599.

Great interest attaches to the possibility of lipoids acting as antigens in inducing the production of complement-fixing antibodies. Since various lipoidal substances serve as extracts for the Wassermann reaction (erroneously designated as "antigens" in this connection) it is commonly believed that they may be antigenic. Warden¹ reports that the bacterial fats are antigenic and that they are even more antigenic and specific than the bactericidal proteins. Meyers² has reported that acetone-insoluble extracts (presumably phosphatids) of tapeworms and tubercle bacilli produce complement-fixing antibodies when injected into rabbits, and Bergel³ has found the same after injecting rabbits with lecithin. Meinicke⁴ has likewise subscribed to the lipoidal nature of the complement-fixation reaction. Against these positive findings are numerous negative reports by Ritchie and Miller,⁵ Kleinschmidt,⁶ Thiele,⁷ and others who were unable to produce complement-fixing antibodies with lipoids. The mere fact that complement fixation in syphilis occurs with lipoidal substances is no criterion of the antigenic activity of lipoids; immunization of rabbits by Fitzgerald and Leathes⁸ with these substances obtained from liver, which were capable of acting as "antigens" in the Wassermann reaction, did not engender the production of complement-fixing antibodies.

While it cannot be stated definitely that lipoids are without the power of stimulating the production of complement-fixing antibodies, the preponderance of evidence is in favor of this view. The presence of even traces of protein in the lipoids employed for immunization may suffice for inducing the production of these antibodies, and a sufficient amount of work with protein free lipoids has not been done to warrant the statement that pure lipoids from bacteria, protozoa, or other sources are true antigens capable of engendering complement-fixing antibodies either during the course of disease or by artificial immunization.

Very little work has been done with the glucosids. The work of Ford and others discussed in previous chapters, indicates that toxic glucosids, and particularly those which may be hydrolyzed by enzymes, may produce antibodies of antitoxic nature, but purified glucosids are probably unable to induce the production of complement-fixing antibodies.

The antigenic activity of glycoproteins has been studied by Elliott⁹ with mucins of ox tendon, ox submaxillary gland, and swine stomach. These were found antigenic, but not to the same degree as the simple proteins. In all probability the antigenic portion of these resided in the protein and not in the carbohydrate group.

Nature of Complement-fixing Antibody (Alexofixagins); Relation to Precipitins, Cytolysins, and Albuminolysins.—In a strict sense hemolysis, bacteriolysis, and other cytolytic reactions are examples of complement fixation in that complement becomes fixed or absorbed by a sensitized antigen (antigen + amboceptor or sensitizer). With the discovery of the complement-fixation reaction by Bordet and Gengou it was concluded that the mechanism was the same, that is, complement was fixed by antigen and antibody and, therefore, was not available for hemolysis when cor-

¹ Jour. Infect. Dis., 1918, 22, 133; *ibid.*, 23, 504; 1919, 24, 285.

² Ztschr. f. Immunitätsf., 1910, 7, 732; 1911, 9, 530; 1912, 14, 355.

³ Deut. Arch. klin. Med., 1912, 106, 47.

⁴ Ztschr. f. Immunitätsf., 1918, 27, 350; 1919, 28, 280.

⁵ Jour. Path. and Bact., 1913, 17, 429.

⁶ Berl. klin. Wchn., 1910, 47, 57.

⁷ Ztschr. f. Immunitätsf., 1913, 16, 160.

⁸ Univ. of Californ. Pub. in Path., 1912, 2, 39.

⁹ Jour. Infect. Dis., 1914, 15, 501.

puscles and hemolysin were subsequently added. According to this view the complement-fixing antibody (which may be designated as *alexofixagin*) is of the nature of amboceptor (called the "Bordet amboceptor"), and the reaction has been advocated as a more sensitive test for bacteriolytic amboceptors in serum than the bacteriolytic tests of Pfeiffer and others described in a preceding chapter.

Neufeld and Haendel,¹ however, have reported that at 0° C. sensitized cholera spirilla fixed hemolytic complement, while at 37° C. they fixed both hemolytic and bacteriolytic complements. They concluded from these experiments that the fixation of complement occurring at or near 0° C. was due to the complement-fixing antibody and that it is distinct from the bacteriolytic amboceptor operative at 37° C.

Neufeld and Hune² have, furthermore, found that bacteriolysins and complement-fixing antibodies in typhoid immune sera were not identical; Haendel³ reported similar findings with typhoid and cholera immune sera, and Torrey⁴ with antigenococcic serum. In other words, while complement-fixing antibodies may be of the nature of amboceptors or sensitizers, there is little or no evidence to support the view that they are identical with the bacteriolysins and proteolysins.

In 1905 Moreschi,⁵ in a study of the mechanism of the anticomplementary activity of serum to be discussed shortly, came to the conclusion that this phenomenon was not due to the development of anticomplements, but to a fixation of complement by precipitate formed by precipitin and homologous antigen. At the same time and independently of Moreschi the investigations of Gay⁶ showed that precipitates may fix complement and that the degree of complement fixation bore a relation to the amount of precipitate formed, and this to the amount of antiserum present.

This explanation, however, was soon challenged and has resulted in a large number of investigations bearing upon the relation of precipitins and precipitates to the phenomenon of complement fixation. Neisser and Sachs,⁷ Muir and Martin,⁸ Friedberger,⁹ Sobernheim,¹⁰ Altmann,¹¹ and others generally observed fixation of complement when precipitation occurred, but likewise noted that complement fixation occurred upon mixture of homologous antigens and antisera when visible precipitation was not apparent.

Dean,¹² however, in a thorough study of the subject came to the conclusion that precipitation and complement fixation occur together, although visible precipitation may not be apparent under ordinary conditions. He believes that the two reactions represent two phases of the same reaction and that "flocculent precipitation represents the final stage of a change which can be recognized in its earliest and incomplete stage by means of a complement-fixation reaction."

The question now arises: Is complement fixation a mere matter of physical absorption of complement by precipitates or do precipitins and a separate complement-fixing antibody occur together or separately in immune sera? Gay¹³ has declared himself in favor of the latter view and that complement-fixing lysins and precipitins are separate and distinct, although likely to occur in protein antisera.

¹ Arb. a. d. kais. Gesund., 1908, 28.

² Arb. a. d. Gesundh., 1907, 25, 164.

³ Deutsch. med. Wchn., 1907, 2030.

⁴ Jour. Med. Res., 1910, 22, 95.

⁵ Deutsch. med. Wchn., 1906, 578.

¹⁰ Centralbl. f. Bakteriöl., Ref., 1906, 38, 114 (Beiheft).

¹¹ Centralbl. f. Bakteriöl., 1910, 54, 174.

¹² Centralbl. f. Bakteriöl., 1912, 13, 84.

⁵ Berl. klin. Wchn., 1905, No. 37.

⁶ Centralbl. f. Bakteriöl., 1905, 93, 603.

⁷ Berl. klin. Wchn., 1905, 1388.

⁸ Jour. Hyg., 1906, 6, 265.

¹³ Univ. of Californ. Publ., 1911, 2.

In the chapter on Precipitins we discussed the nature of these antibodies in relation to amboceptors (proteolysins or albuminolysins) according to the results of the investigations of Zinsser¹ who regards them as identical. In relation to the nature of complement-fixing antibodies Zinsser believes that the precipitins unite with the antigen forming a complement-fixing complex and that since both complement and sensitized antigen are colloidal in nature, they follow the laws governing other mutually precipitating colloids and precipitation occurs only as a purely secondary phenomenon when concentrations lie within the proper zones.

Undoubtedly precipitation bears an important relation to complement fixation not only in reactions by soluble proteins, as sera and milk and their antisera, but likewise in complement-fixation reactions in syphilis, which are the most pronounced reactions of this kind. Whether precipitation is always caused by precipitins and especially in relation to the Wassermann reaction is an open question. Precipitins bear a close relationship to proteolysins, but the nature of this relationship is not clear. Precipitation appears to be an integral part of the complement-fixation reaction with cells, soluble proteins and in syphilis and frambesia, but whether precipitation is brought about by precipitins or is the result of physicochemic changes in serum and antigen by a separate and peculiar complement-fixing antibody cannot be definitely stated. Complement fixation in syphilis and frambesia is particularly complex and is discussed separately in the following paragraphs.

Specificity of Complement-fixing Antibodies (Alexofixagins).—These antibodies possess an extremely high degree of biologic specificity for the antigen engendering their production. First discovered in relation to bacterial infections, the complement-fixation test has proved a reliable and sensitive means for the diagnosis of some infections and for differentiating among those substances capable of acting as alexofixagins, that is, capable of stimulating the tissues with the production of a complement-fixing antibody or alexofixagin. Some micro-organisms, as the pneumococcus, engender very little complement-fixing antibody; others, as the Gram-negative bacilli and particularly those of the typhoid-colon-dysentery-cholera group, induce the production of these antibodies to a well-marked degree. *Under proper technical conditions* the complement-fixation reaction is a more accurate means for studying biologic relationships than the agglutination and precipitin reactions.

The same may be said of the alexofixagins and complement-fixation reaction with soluble proteins of animal and plant origin, as sera, milks, extracts of meats, etc. The Wassermann reaction in syphilis and frambesia tropica is the only exception to this general rule of biologic specificity on the part of alexofixagins and the fixation reaction.

The Nature and Specificity of the Spirochetic Complement-fixing Antibody (Syphilis "Reagin").—The unique character of the complement-fixation reaction in syphilis and frambesia tropica requires that its nature, specificity, and mechanism be discussed separately from other complement-fixation reactions. When first discovered by Wassermann, Neisser and Bruck, and independently by Detre, it was considered a fixation of complement by syphilis amboceptor and extracts of *Treponema pallida* contained in the syphilitic tissues employed for the preparation of antigens; in other words, a direct application of the Bordet-Gengou phenomenon. With the discovery that "antigens" for this complement-fixation reaction may be prepared of non-syphilitic tissues, the whole nature and mechanism of the

¹ Jour. Exper. Med., 1912, 15, 529; *ibid.*, 1913, 18, 219.

reaction in syphilis became sharply differentiated from other complement-fixation reactions because the reaction could no longer be regarded as biologically specific.

As is now well known, the lipoids¹ have always been chiefly concerned in the reaction, although Wassermann and Detre and their co-workers naturally ascribed the complement-fixing powers of their extracts to the presence of the *Treponema pallidum*. It is, indeed, fortunate that pure cultures of the *treponema* were not available at the time the original studies were made, for these would naturally have been employed as antigen, and as subsequent work with pallidum antigens has shown complement fixation to be quite irregular and less reliable than when lipoidal tissue extracts are used, this result, coupled with the imperfect understanding and faulty technic of the earlier investigations, would probably have yielded results so discouraging as to constitute weighty drawbacks to the full development of the reaction.

Notwithstanding the large amount of work that has been done in an effort to ascertain the true nature of the syphilitic reaction, a correct explanation of its mechanism is still lacking, as the large number of theories advanced tend to show.

While lipoidal extracts, as well as normal and luetic serums, may separately absorb or fix small amounts of complement, a mixture of a suitable extract and syphilitic serum is capable of fixing large amounts of complement, and this constitutes the main principle and all that is definitely known of the syphilitic reaction.

The serum of a syphilitic is characterized, therefore, by the presence of this lipodotropic, antibody-like substance, called "reagin" by Neisser, which has a great affinity for lipoids and in mixture with them will cause the absorption or fixation of complement to a well-marked degree. Instead of being an example of complement fixation in a mixture of specific antigen with specific antibody, as originally believed, it is technically a non-specific reaction, but practically it is highly specific, since this peculiar antibody is found in largest amount and most constantly in syphilis, and to a lesser extent in practically only one other disease, namely, frambesia. In countries and districts where this disease is infrequent or unknown the reaction for syphilis is highly specific.

As will be shown further on, the presence of this lipodotropic substance is dependent upon the activities of the *Treponema pallidum*, and when repeated tests continue to show its presence, there is every reason to believe that a cure has not been effected, but that the patient still harbors the living parasite.

Citron has advanced the hypothesis that the antibody-producing antigen is a toxolipoid, which would explain the fact that while pure lipoids, such as lecithin, cannot stimulate antibodies (Bruck), as the toxolipoid does, they can, nevertheless, react with the lipodotropic antibodies *in vitro*, with fixation or absorption of complement. As Sachs and Altman point out, an equally tenable theory would be that in syphilis the tissues undergo such alterations that they can produce antibodies to the lipid substances as may be contained in the spirochetes themselves.

While the production of this lipodotropic antibody is still unexplained, the fact remains, nevertheless, that it forms the basis of the biologic syphilitic reaction, and in a mixture with a suitable lipid is capable of absorbing or inactivating complement to a marked degree. Whether or not it is a true

¹ The term "lipoid" ("fat-like") is applied to compounds that are soluble in ether, alcohol, chloroform, and benzol, but every lipid is not soluble in all these reagents.

antibody in the sense that it is inimical to the spirochete is doubtful; by many it is regarded as a secondary product of cellular activity, and, as stated above, has been called syphilis "reagin."

Investigators in this field anxiously awaited the isolation of the *Treponema pallidum* in pure culture, believing that if this result were secured it would be possible to work with a specific antigen, determine the nature of the true syphilis antibody, and possibly establish a complement-fixation test specific for syphilis.

In 1909 Schereschewsky,¹ using an antigen of an impure and non-pathogenic culture of a spirochete regarded as the *Treponema pallidum*, reported positive complement-fixation reactions with the majority of serums tested.

In 1912 Noguchi,² having undoubtedly isolated the spirochete in pure culture, prepared antigens and found that, whereas certain long-standing or treated cases of syphilis yielded positive reactions with the pallidum antigens, the reactions were uniformly negative when the lipoidal extracts were used. In primary and secondary syphilis the reactions with pallidum antigens were uniformly negative, whereas with the lipoidal extracts they were uniformly positive. As a result of his experiments Noguchi concluded that in syphilis there is produced a true antibody that reacts specifically with pallidum antigen, in addition to the lipodotropic "reagin," which reacts with lipoidal extracts, and whereas the latter indicates activity of the infecting agent, the former is a gage of the defensive activity of the infected host.

Craig and Nichols,³ using alcoholic extracts of pure cultures in ascite-kidney agar of *Treponema pallidum*, *Spirochete pertenuis*, and *S. microdentium*, found similar positive reactions in all stages of syphilis with the three antigens, but the reactions were weaker and less constant as compared with those obtained with a stock of lipoidal extract.

Similar studies conducted by Kolmer, Williams, and Laubaugh⁴ with aqueous and alcoholic extracts of pallidum cultures showed positive reactions in secondary, tertiary, and congenital syphilis. The aqueous extracts yielded better reactions than the alcoholic extracts; in practically all instances, however, the reactions were weaker than those obtained with the ordinary tissue extracts. Control antigens of typhoid and cholera bacilli and sterile culture-mediums demonstrated that all contained lipoidal substances that may give weak reactions with the lipophilic syphilis "reagin." This may explain Schereschewsky's positive reactions with an antigen of a spirochete that in all probability was *Spirochete microdentium* (Noguchi).

The true nature of the Wassermann-Detre reaction, therefore, cannot be said to have been determined. It is probable that the ordinary syphilis reaction is in itself not dependent upon a true antibody, and that the reaction is not an immunity reaction, but due rather to the presence of peculiar tissue products (reagins) altered by the presence and activities of the spirochetes themselves, and that the Wassermann reaction is an expression of this active injury to tissue cells. In addition to this secondary product there is probably a true syphilis antibody that may yield specific complement fixation with pallidum antigens.

In so far as the Wassermann reaction is concerned, the true antibody is entirely secondary in importance, and the whole question is intimately concerned with the chemistry of lipoids. While future researches in im-

¹ Deutsch. med. Wchn., 1909, xxv, 1652.

² Jour. Amer. Med. Assoc., 1912, lviii, 1163.

³ Jour. Exper. Med., 1912, xvi, 336.

⁴ Jour. Med. Res., 1913, xxviii, 345.

munochemistry may reveal the mechanism of the reaction, the principles are at least well understood at present, so that the syphilis reaction is proving of great diagnostic and practical value.

Mason¹ has also recently discussed the mechanism of the Wassermann reaction in relation to colloidal reactions, believing that the reaction may in some way depend on certain physical differences in the sera as shown by the increased flocculability of syphilitic serum in the presence of certain colloidal solutions.

Mechanism of Complement Fixation.—The divergent views of Bordet and Ehrlich on the mechanism of antigen-amboceptor action have been given elsewhere. Bordet believes that the antibody unites directly with the antigen, and serves to sensitize and prepare it for direct union with the alexin or complement in a manner similar to that of using a mordant in aiding the penetration of a dye-stuff. In the absence of the homologous and specific antibody (sensitizer) the antigen is incapable of absorbing more than very small amounts of complement or none at all. In the absence of antigen the sensitizer and complement do not unite, or unite to but a very slight degree. The important requirement for complement fixation is, therefore, an antigen that has been sensitized by the antibody, and in this manner has an increased combining affinity for complement.

According to Ehrlich and Morgenroth, however, the complement does not unite directly with the antigen, but only indirectly through the antibody, which acts as a connecting link or amboceptor between antigen and complement. Antigen alone, or even amboceptor alone, binds the complement only very slightly or not at all. The important requirement for complement fixation is an amboceptor attached to its homologous or suitable antigen, which increases the affinity and fixing power of the amboceptor for the complement (see Fig. 85).

Complement Fixation a Colloidal Reaction.—Many observations support the view that complement fixation by a specific antigen and its antibody is really complement absorption by a precipitate that forms when antigen and antibody are mixed. As previously stated, all antigens are probably protein in character. While there is some evidence to show that lipoids, and even carbohydrates, may act as antigens, there is no doubt that the chief antigenic principles of any antigen are of protein structure; hence when mixed with an immune serum containing specific antibodies, it is believed that an invisible precipitate is formed that absorbs the complement. With serum antigens the quantity of protein is so large that a precipitate can readily be seen (the precipitin test). A serum antigen and its antibody may, however, be so highly diluted that, when mixed, a precipitate is not visible, although complement may be fixed (complement-fixation test for the differentiation of proteins). Moreschi and Gay have contended for many years that complements may become entangled and absorbed in such precipitates. Reasoning on the basis of the colloidal theory, it is possible that transition compounds of very diverse nature are formed when antigen, antibody, and a complement are mixed. This view concerning the action of complements and anticomplements is supported by numerous investigators who have examined the question from the standpoint of colloidal reactions. Thus in a complement-fixation test a mixture of antigen, antibody, and a complement in definite proportions results in the formation of new compounds of opposite electric charge, which tend to aggregate in masses (although these may be so small as to be invisible) and reduce their surface tension in just the same manner as agglutination

¹ Bull. Johns Hopkins Hosp., 1920, 31, 234.

and precipitation are brought about after the colloidal theories. When corpuscles and hemolytic antibody are subsequently added hemolysis does not occur because free complement is absent.

A process similar to complement absorption by a specific antigen and its antibody is the *Wassermann reaction*. According to the colloidal theories, this reaction may be explained as due to the formation of an invisible precipitate by interaction between some substance in the serum of a luetic person (probably in the nature of an altered globulin), complement, and lipoidal substances contained in an alcoholic or watery extract of a normal or a diseased organ. This view is supported by the fact that euglobulin is known to be generally increased in the body fluids of syphilitics, and by analogy with the various flocculation tests that have been devised for the diagnosis of syphilis, as, for example, the reaction of Porges and Meier, Meinicke, Sachs and Georgi, Vernes, etc., which are dependent upon the appearance of a precipitate when luetic serum is mixed with an emulsion of lecithin, sodium glycocholate, alcoholic extracts of tissues, etc. The exact nature of the antibody in syphilitic serums that forms these new compounds with lipoids and complement, resulting probably in the absorption of complement, is unknown. It is most likely in the nature of a globulin, its main characteristic being the power it possesses of reacting with lipoids. Schmidt¹ ascribes the reaction to the physicochemic properties of the globulins of the syphilitic serum, which he believes possess a greater affinity for the colloids of the antigen than do normal globulins. This view is supported by the common observation that the turbidity of the antigen emulsion is closely related to its efficiency, since clear solutions are less active.

THE ANTICOMPLEMENTARY ACTIVITY OF ANTIGENS AND SERA

Practically all substances employed as antigens for the immunization of animals and the complement-fixation test, including the living micro-parasites producing disease and antibody production, are capable of absorbing, fixing, or inactivating hemolytic complement in the test-tube independent of the presence or absence of specific complement-fixing antibodies. The degree of this complement absorption or fixation varies greatly with the kind and amount of different antigens, but is so important in connection with the complement-fixation test that this property of the substance being employed as antigen must be carefully determined by a process of titration.

Under certain conditions all sera and other body fluids employed in the complement-fixation test and irrespective of whether or not they are normal or immune, may possess or acquire a similar property for absorbing, fixing, or inactivating complement by themselves and in the absence of antigen. This property is likewise so important in relation to the practice of the complement-fixation test that controls on this property of each serum being examined are always included (the serum controls).

The presence of this property of antigens, sera, and other substances is detected by the interference of hemolysis when they are placed in test-tubes along with complement followed after a suitable period by the addition of corpuscles and specific hemolysin. Hemolysis is absent or incomplete because of the absorption, fixation, or destruction of all or a portion of the complement. Hence the process has been designated as the *anticomplementary activity* of these substances.

The phenomenon may be divided into two entirely different processes,

¹ Ztschr. f. Hygiene, 1911, 69, 513.

namely, (a) destruction or interference of activity of complement, and (b) absorption or fixation of complement. In the test-tube, however, this distinction may not be detectable because the end-result of absent or incomplete hemolysis is the same for both.

Destruction of complement or interference with its activity is readily understood and may be caused by such factors as changes in the hydrogen ion concentration of sera (acids) brought about by autolytic changes and the activity of ferments in the sera or furnished by contaminating bacteria. In bacterial antigens prepared with nutrient broth, the latter may contain various acids, alkalis, or protein split products which are known to be very potent in the inactivation and destruction of complement. In the alcoholic or salt solution extracts of tissues employed as "antigens" in the Wassermann test the anticomplementary activity may be caused by various lipoidal constituents, protein substances, or even the alcohol itself. Very probably the *anticomplementary activity of lipoids, carbohydrates, and unorganized substances is due to actual destruction of complement or interference with its activity.*

Aside from the destruction or inactivation of complement due to changes in its active portion, it would appear that sera, suspensions of bacteria, and other cells and extracts of these as well as solutions of proteins of higher plants, are able to absorb or fix complement in the absence of homologous antibodies.

These effects are sometimes designated as non-specific complement-fixation reactions, but I believe it is well to regard them as examples of anticomplementary action. As will be discussed in the succeeding section normal human, rabbit-, dog-, mule-, and other sera of the lower animals may not be anticomplementary in ordinary amounts, but when set up in complement-fixation tests with antigens composed of non-anticomplementary amounts of bacterial extracts or alcoholic extracts of tissues, may yield strongly positive complement-fixation reactions. I believe these are properly designated as examples of non-specific complement fixation.

Kinds of Anticomplementary Substances (Antilysins).—As previously stated, many substances employed in complement-fixation tests may prove to be anticomplementary (also called "antilytic") if they are employed in sufficiently large amounts; these antilysins may be found in:

1. Suspensions and extracts of bacteria as shown by Wilde.¹ All bacterial antigens are anticomplementary, although they vary greatly in the amounts required to show these effects. Bacteria growing in serum render the latter highly anticomplementary, as shown by Craig² and others.

2. Extracts of tissues, as shown by von Dungern³ and tissue constituents, as shown by Levene and Baldwin.⁴ These include the watery, saline, and alcoholic extracts of tissues commonly employed as "antigens" in the Wassermann test.

3. Sera of various animals. Fresh sera are generally free of anticomplementary substances. Sterile sera may become anticomplementary and especially when kept at 20° to 38° C., but these substances are generally removed by heating to 55° C. for thirty minutes. Sera deeply tinged with hemoglobin are apt to become anticomplementary. Sera contaminated with bacteria become highly anticomplementary. Heating the sera of some animals to 60° C. and higher renders them anticomplementary and I shall refer to this subject shortly in more detail.

Certain pathologic conditions may render serum anticomplementary,

¹ Berl. klin. Wchn., 1901, 38, 878.

² Jour. Exper. Med., 1911, 13, 521.

³ Münch. med. Wchn., 1900, xlvii, 677.

⁴ Jour. Med. Research, 1904, 12, 205.

notably uremia, as shown by Neisser and Doring,¹ Neisser and Friedemann,² Bergmann and Keuthe,³ Laquer,⁴ and others.

Absorption of sera by corpuscles to remove hemolysins or absorption by bacteria to remove agglutinins, opsonins, and bacteriolysins may render them anticomplementary; these antilysins are generally removed by heating the sera to 55° C. for thirty minutes.

4. Precipitates may absorb complement and simulate the effects of anticomplementary sera as shown by Moreschi⁵ in a study of the anticomplements of sera. I will later refer to these in a discussion of the mechanism of action of antilysins.

The Influence of Heat Upon Anticomplementary Substances (Thermolabile and Thermostabile Antilysins); General Properties.—Human sera may prove anticomplementary when unheated, but lose this property completely or partly after heating in a water-bath to 56° C. for fifteen to thirty minutes; these antilysins are designated as *thermolabile* and to remove them is one reason why sera are heated routinely for the Wassermann and other complement-fixation tests. The antilysins may not be influenced by heating to 56° C., and these are known as *thermostabile*. According to Manniger⁶ horse-sera must be heated to 56° C. for thirty minutes and the sera of mules and asses to 63° to 64° C. for thirty to forty minutes to remove the thermolabile antilysins.

Heating to 56° C. usually has very slight influence upon the anticomplementary effects of bacterial suspensions, and extracts and tissue extracts employed as antigens in complement-fixation tests; these antilysins are usually of the thermostabile variety.

Curiously, heating the sera of some animals is said to develop in them anticomplementary effects. Camus and Gley⁷ found this true of eel-sera heated to 56° C.; Ehrlich and Sachs⁸ observed that heating dog- and ox-serum to 60° C. had the same effects, and Sachs⁹ has described similar changes in rabbit-serum. Noguchi¹⁰ has observed that heating normal sera of the dog, sheep, and ox to 56° C. results in the development of anticomplementary activity.

Anticomplementary substances are non-specific, that is, when present in a serum they are likely to interfere with hemolysis in different hemolytic systems.

Noguchi has observed that absorption of dog-sera with corpuscles may remove the thermostabile antilysins, but Kyotoku¹¹ was unable to accomplish this in my laboratory working with anticomplementary human sera. Absorption with barium sulphate, kaolin, bone, ash, and charcoal removed them to a slight degree. Filtration of diluted human sera through Kitasato filters was found to remove the antilysins; Kyotoku noted but slight influence of filtration upon the antibodies, but I have since observed that a large portion of the complement-fixing antibodies are likewise removed by this process. As far as I know there is no method for removal of thermostabile antilysins from sera which does not remove completely or partly the antibodies at the same time.

The Chemical Nature of Anticomplementary Substances.—The blood lipoids have been observed by many investigators to possess antihemolytic

¹ Berl. klin. Wchn., 1901, 38, 593.

² Berl. klin. Wchn., 1902, 39, 677.

³ Ztschr. f. exper. Path. und Therapie, 1906, 3, 255.

⁴ Deutsch. med. Wchn., 1901, 27, 744.

⁵ Berl. klin. Wchn., 1902, 39, 492.

⁶ Berl. klin. Wchn., 1905, xlii, 1181.

⁷ Deutsch. med. Wchn., 1905, 31, 705.

⁸ Ztschr. f. Immunitätsf., 1921, 31, 222.

⁹ Jour. Immunology, 1919, 4, 239.

¹⁰ Compt. rend. Soc. de biol., 1901, liii, 732.

¹¹ Jour. Immunology, 1919, 4, 239.

properties. Landsteiner and v. Eisler¹ and Bang and Forssmann² claimed to have secured them by ether extraction of corpuscles and serum; Noguchi³ has likewise reported that ether extracts of blood-serum and corpuscles, freed from lecithin and certain related bodies, contains the thermostable antilysin in concentrated but not pure form, which can be dissolved in saline solution and to which solution he has applied the name "protectin." Zinsser and Johnson⁴ were unable to extract the thermolabile antilysins of human sera with ether, but found that they were removed by precipitation of the globulins. With normal rabbit- and dog-sera I have observed that a portion of the antilysins were removed by extraction with ether and chloroform, but that both the globulin and albumin fractions were likewise concerned and especially the globulins.⁵ Kyotoku in his study with human sera, was likewise unable to remove the antilysins with ether and found them closely allied with the protein constituents and especially the globulin fraction. Manniger has⁶ likewise identified the antilysins of the sera of horses, mules, and asses with the globulins of serum.

It is well established that serum cholesterol is antihemolytic not only by effect upon the complement in serum hemolysis, but likewise upon the hemotoxins of bacteria, venom, saponin, etc. It is highly probable that the antilysins of human and other sera are identified with the protein fractions under certain circumstances and with the lipoids in others. They appear to be a group of substances capable of affecting complement. Their exact chemical nature is as yet unknown and the subject urgently requires further investigation.

The Mechanism of Action of Anticomplementary Substances.—As first shown by Gay⁷ and Moreschi⁸ the anticomplementary activity of serum may not be caused by the development of antilytic substances, but be the result of complement absorption or fixation by the development of precipitates. For example, Sachs has showed that when sheep corpuscles are added to inactive rabbit-antisheep serum for the absorption of hemolysin that the serum may become anticomplementary. According to Gay this is caused by traces of sheep-serum adhering to the corpuscles producing precipitates with antisheep precipitin likewise present in the immune serum. Not infrequently the addition of washed sheep corpuscles to human serum for the absorption of natural antisheep hemolysin renders the serum anticomplementary. The anticomplementary activity is removable, however, by heating the serum to 55° C. for fifteen to thirty minutes. Whether these thermolabile anticomplementary effects are due to precipitates I am unable to state, but doubt that such is the case.

The anticomplementary effects of suspensions or extracts of bacteria and tissue cells may likewise be caused by the development of invisible amounts of precipitate capable of absorbing complement due to the presence of natural precipitins in the complement serum acting with the bacterial or other cellular protein; or it may be that natural amboceptors for these bacterial or cellular substances are present in the complement serum, resulting in the specific fixation of complement and simulating the effects of an anticomplementary action.

¹ Wien. klin. Wchn., 1904, 27, 676; Centralbl. f. Bakteriöl., 1905, 39, 309.

² Centralbl. f. Bakteriöl., 1905, xl, 150.

³ Jour. Exper. Med., 1906, 8, 726.

⁴ Jour. Exper. Med., 1911, 13, 31.

⁵ Jour. Infect. Dis., 1916, 18, 46.

⁶ Ztschr. f. Immunitätsf., 1921, 31, 222.

⁷ Centralbl. f. Bakteriöl., 1905, 39, 603.

⁸ Berl. klin. Wchn., 1905, xlii, 1181.

The lipid antilynsins are probably in large part composed of cholesterol and may inhibit hemolysis by directly neutralizing or destroying complement or, according to Noguchi, by increasing the resistance of corpuscles to hemolysis.

In some instances at least the anticomplementary action of sera, bacterial suspensions and extracts, and alcoholic extracts of tissues may be due to physical conditions of a colloidal nature. Manniger believes that the serum globulins are responsible for anticomplementary effects by absorbing complement, and that the removal of this property by heating serum is due to the formation of compound of globulin and water, resulting in the production of an emulsoid colloid without ability to absorb complement. It may be that the anticomplementary effects of bacterial antigens are due to the absorption of complement by fine particles comparable to the antilytic effects of such inorganic emulsions as quartz, sand, and kaolin.

Importance of Anticomplementary Action of Sera and Antigens in Relation to Complement-fixation Tests and Reactions.—As previously stated, antilynsins in serum or antigen employed in a complement-fixation test may interfere with hemolysis and thereby resemble a specific complement-fixation reaction. For this reason every substance employed as antigen must be titrated for its anticomplementary effects; the smallest amount that just begins to interfere with hemolysis is known as the *anticomplementary unit*, and in the main tests the antigen is used in a small fraction of this amount in order to avoid non-specific reactions.

The anticomplementary activity of an antigen is determined by placing increasing amounts in a series of test-tubes with a fixed amount of complement, and incubating the mixtures for the same length of time and at the same temperature as employed in the main tests. Hemolysin and corpuscles are now added to each tube and the mixtures reincubated.

The occurrence and degree of hemolysis is then read and indicate the presence or absence of anticomplementary effects in the range of doses of antigen employed in the titration. The details of technic will be described in the succeeding chapters.

Serum controls are included in every complement-fixation test to determine whether or not the serum in the amount employed is free of anticomplementary effects. If hemolysis is incomplete with a satisfactory hemolytic system, the inference is that the serum contained antilynsins and the results of the complement-fixation test should be interpreted with great care and, better, discarded, with a repetition of the tests with fresh sera.

By referring to the original Bordet experiment it will be observed that this investigator controlled any non-specific absorption of complement by both the immune and the normal serum in tubes C and D of the series by using the full dose of these serums with a similar amount of complement, and noting that hemolysis was complete. His controls, E and F, were to determine if the process of inactivation or removal of native complement from the two serums was complete, and the total absence of hemolysis showed that it was. His control on the anticomplementary action of the antigen was also included in tube D, for if the emulsion alone had absorbed complement to any degree, hemolysis would have been incomplete.

NON-SPECIFIC COMPLEMENT FIXATION

Of considerable interest and practical importance is the possibility of normal human sera and those of some of the lower animals of yielding non-specific complement-fixation reactions with various bacterial and tissue

antigens. Both sera and antigens are free of anticomplementary effects in these reactions in the amounts employed, and for this reason the phenomenon is to be separated from the effects of the antilysins previously discussed.

Non-specific Complement Fixation by Normal Human Sera (Proteotropic Reactions).—When *unheated* human sera are employed in the Wassermann test with alcoholic extracts of tissues for “antigens,” positive reactions may occur with sera from non-syphilitic individuals. Seligman and Pinkus,¹ Noguchi,² Browning and McKenzie,³ and others have drawn attention to this phenomenon, and Noguchi believes that it is caused by the presence of protein in the alcoholic extract of tissue being employed for “antigen.” For this reason he has designated the reaction as *proteotropic complement fixation*. *Heated human sera do not yield these reactions*. When unheated sera are employed in a complement-fixation test for syphilis, the tissue extract antigen should be free of protein, and for this reason Noguchi devised a method for preparing an extract of alcohol-soluble but acetone-insoluble lipoids which are to be preferred to plain or crude alcoholic extracts for tests of this kind, inasmuch as the latter usually contain protein. In my experience the use of properly prepared acetone-insoluble lipoids reduces the percentage of pseudopositive or proteotropic reactions to less than 2 per cent., and this kind of “antigen” is to be preferred in all complement-fixation tests employing unheated serum;⁴ heating to 55° C. for fifteen minutes has been found sufficient for removing this property from human sera.⁵

Non-specific Complement Fixation by Normal Rabbit-, Dog-, and Mule-sera.—*The fresh normal sera of several species of animals may absorb or fix complement with various lipoidal and bacterial antigens in a non-specific manner*. Schilling and Hoesslin⁶ were probably among the first investigators to note this phenomenon with normal rabbit-serum. Manteufel and Woithe⁷ and Browning and McKenzie⁸ have also noted the phenomenon during studies in experimental trypanosomiasis. Dohi⁹ examined the sera of 74 normal rabbits, using as antigen an alcoholic extract of syphilitic liver, and found that 39 reacted positively and 35 negatively. He also observed that heated serum was more likely to show this non-specific absorption of complement than unheated serum. Browning and McKenzie, however, state that they have not observed this phenomenon when using serum in a fresh, or active, condition. Blumenthal¹⁰ has likewise observed positive reactions with normal rabbit-serum, using an alcoholic extract of syphilitic liver as antigen; Craig and Nichols,¹¹ on the other hand, have reported uniformly negative results with an alcoholic extract of syphilitic liver. Similar observations on this power of normal rabbit-serum to absorb complement in the presence of lipoidal antigens have been made by Emmanuel¹² and by Epstein and Pribram,¹³ the former stating that the administration of salvarsan removes this property temporarily, and the

¹ Ztschr. f. Immunitätsf., 1910, 5, 377.

² Serum Diagnosis of Syphilis, Lippincott Co.

³ Diagnosis and Treatment of Syphilis, Lea & Febiger, 1913, 20.

⁴ Jour. Immunology, 1916, 2, 23.

⁵ Amer. Jour. Syph., 1920, 4, 641.

⁶ Deutsch. med. Wchn., 1908, 34, 1422.

⁷ Centralb. f. Bakteriöl., R., 1909, 43, 359.

⁸ Jour. Path. and Bacteriol., 1909–11, 15, 182.

⁹ Beitr. z. path. u. Therap. der Syph., 1911, 514.

¹⁰ Berl. klin. Wchn., 1911, 48, 1462.

¹¹ Jour. Exper. Med., 1911, 14, 206.

¹² Berl. klin. Wchn., 1911, 48, 2335.

¹³ Ztschr. f. exper. Path. u. Therap., 1909, 7, 549.

latter making similar claims for the mercurials. Casselman and myself¹ observed a large percentage of positive reactions with the sera of 117 normal rabbits and various lipoidal extracts used as antigens in the Wassermann reaction. In a further study Miss Trist and myself² found that while heated rabbit-serum yielded positive reactions with 38 to 49 per cent. of sera with lipoidal antigens, active or unheated sera yielded but 5 to 15 per cent. positive reactions. Bacterial antigens yielded even higher percentages of positive reactions. Similar results have been reported by Kritchewsky,³ Hellens,⁴ and Huddleson.⁵ Pearce and myself⁶ have also found that the sera of animals reacting positively or negatively generally continue to react in the same manner over long periods of observation. Rossi,⁷ Miss Trist and myself⁸ have also found that the sera of a large percentage of normal dogs tends to yield non-specific fixation with various lipoidal and bacterial antigens. The mechanism of this phenomenon is not understood; it would appear that the complement-absorbing body is in both the serum lipoids and proteins.⁹ In my experience heating these sera at 56° C. for half an hour greatly increases their property for non-specific fixation of complement; heating at 62° C. for the same period lessens the tendency.¹⁰ *The subject is of great importance in view of the frequency with which complement-fixation studies are conducted with rabbit-, dog-, and mule-sera.*

QUANTITATIVE FACTORS IN COMPLEMENT-FIXATION TESTS

From what has been said it will, therefore, readily be appreciated that complement-fixation tests are largely quantitative. Equally fallacious results may be obtained by using too large or too small amounts of the various ingredients.

Necessity for Using Optimum Amount of Antigen.—While it is possible to use too large quantities of antigen, so that non-specific absorption of complement occurs, leading to false positive reactions, it is also possible to use an amount so small that any specific absorption of complement by antigen and antibody cannot readily be detected. The proper amount to use must be determined by titration.

The same is true, but to a much less extent, of the serum being tested, for while too large amounts of serum may lead to non-specific fixation of complement, surprisingly small amounts may give well-marked specific fixation, this factor depending, of course, upon the quantity of antibodies contained in the serum.

Necessity for Proper Adjustment of the Hemolytic System.—Of even greater importance are the quantity of complement employed and the proper adjustment of the *hemolytic system*, composed of complement, hemolysin, and corpuscles.

Too large an amount of complement may furnish sufficient to satisfy the complement-fixing antibodies of an immune serum united with the antigen, with enough free complement left over to produce partial or complete hemolysis when corpuscles and hemolysin are subsequently added. In this manner specific complement fixation would be overlooked and a false negative reaction secured.

It is also possible to use too small an amount of complement, with relatively large doses of serum and antigen, so that the complement becomes

¹ Jour. Med. Research, 1913, 28, 369.

² Jour. Infect. Dis., 1916, 18, 20.

³ Ztschr. f. Immunitätsf., 1914, 20, 238.

⁴ Ztschr. f. Immunitätsf., 1913, 17, 156.

⁵ Jour. Immunology, 1916, 2, 147.

⁶ Jour. Infect. Dis., 1916, 18, 32.

⁷ Ztschr. f. Immunitätsf., R., 1909, 1, 429.

⁸ Jour. Infect. Dis., 1916, 18, 27.

⁹ Jour. Infect. Dis., 1916, 18, 46.

¹⁰ Jour. Infect. Dis., 1916, 18, 64.

unduly susceptible to non-specific fixation, and consequently false positive reactions may be secured.

It has previously been explained that an excess of hemolysin may offset any slight deficiency in the amount of complement. For instance, if a small amount of complement is specifically fixed by an antigen and its amboceptor, the addition of too large an amount of hemolysin may result in complete hemolysis of the corpuscles, and thus overshadow the slight but specific fixation of complement.

On the other hand, hemolysis cannot be complete if the dose of hemolysin is too small. With a given dose of corpuscles and complement a certain amount of hemolysin is necessary to produce hemolysis, this dose being determined by a process of titration, as described in a previous chapter. If less than this dose is used, but the amounts of corpuscles and complement remain the same, hemolysis will be correspondingly incomplete and lead to false positive reactions.

A very important feature of all complement-fixation tests will be seen to be a *proper and accurate adjustment of the hemolytic system*. Taking arbitrary amounts of corpuscles and hemolysin as constants, the quantity of complement necessary to produce hemolysis may be determined (titration of complement); or, taking corpuscles and complement as constants, the amount of hemolysin necessary to effect complete hemolysis may be determined. One or the other or both titrations should be made before the main test is attempted, in order to avoid using an excess or too little of either ingredient. If the exact unit of complement and hemolysin are used, the results must be very carefully guarded, because in a general way all antigens and serums exert a slight anticomplementary action that may yield results that will be interpreted as weak positive reactions. For this reason the original complement-fixation tests invariably called for a slight excess of complement or hemolysin, or both, to allow for possible non-specific complement fixation, and this is a good general rule that makes the reaction somewhat less delicate, but more reliable in the long run, especially for inexperienced workers.

Complements of different species of animals act differently in activating a hemolytic amboceptor and toward fixation by antigen-antibody combinations. For instance, a complement from one animal may readily enough combine with a hemolytic amboceptor to produce hemolysis, but will not lend itself for fixation, and is, therefore, unfit for complement-fixation tests. Noguchi and Bronfenbrenner have found guinea-pig-serum most suitable from all standpoints, but it is important to remember that the complementary activity of the serums from different guinea-pigs varies, and, therefore, it is necessary to titrate each complement serum or hemolysin, *i. e.*, adjust the hemolytic system, before the main test is conducted.

These quantitative factors are of great importance, and complicate any complement-fixation method, but efforts to circumvent or ignore them are likely to lead to errors in technic. A proper understanding and appreciation of these factors constitutes the basis for reliable work, whereas less essential details may be altered to conform to the ideas and convenience of the individual worker.

PRACTICAL APPLICATIONS

It will be understood, therefore, that complement-fixation reactions may serve two primary purposes:

1. With a known antigen, the antibody may be found. This is the

usual order in diagnostic tests. For example, in the reaction for syphilis the antigen is furnished and the antibody sought for in the body fluids. So specific has this test proved in the diagnosis of this disease that a positive reaction secured with a proper technic is regarded as strong evidence of the existence of lues, even though the primary lesion had occurred years before and the person is at the time in apparent good health. In the gonococcus fixation test and other tests of a similar nature the antigen is known and is furnished, and the antibody is tested for in the serum.

2. With a known antibody the corresponding antigen may be found. This order of events has less practical application, and is used principally in the diagnosis of blood-stains and in the differentiation of proteins in general. It is also used in making special bacteriologic investigations, when an organism may be identified by specific complement fixation with its known antibody serum. In these instances the antibody serum is secured by immunizing rabbits with a known antigen, the immune serum then being used for selecting the antigen in unknown substances and mixtures.

Complement-fixation methods have their greatest value, and are probably best known, in the serum diagnosis of syphilis—the biologic syphilitic reaction of Wassermann, Neisser and Bruck, and Detre. Although originally believed to be a direct application of the specific Bordet-Gengou phenomenon of complement fixation, subsequent investigations have shown that the antigen need not be specific, in the sense of containing the *Spirocheta pallida*, but that lipoidal substances in general may serve as “antigen,” the peculiar and specific character of the reaction depending upon the nature of the antibody, which has a strong affinity for lipoids, and in such a mixture is capable of absorbing or fixing a considerable amount of complement.

In no other disease has the method been so widely employed as in syphilis, although it possesses value in the serum diagnosis of various bacterial infections, such as gonorrhea, glanders, typhoid fever, echinococcus disease, etc., and in the diagnosis of blood-stains and in the differentiation of proteins in general.

In the following chapter the Wassermann syphilitic reaction will be considered in some detail, as a thorough working knowledge of this test is of great value, and serves as the foundation of complement-fixation technic in general.

CHAPTER XXIII

COMPLEMENT FIXATION IN SYPHILIS

THE TECHNIC AND PRACTICAL VALUE OF THE WASSERMANN AND OTHER COMPLEMENT-FIXATION TESTS

The Phrase "Wassermann Test."—Since the application of the Bordet-Gengou phenomenon to the diagnosis of syphilis by Wassermann, Neisser and Bruck and Detre, many advances have been made in our knowledge of the properties of the several biologic reagents employed and numerous modifications of the original test have been proposed. The phrase "Wassermann test" is used by most persons as a short term for the phrase "complement-fixation test for syphilis," but this is an error and the term should be limited to the test conducted after the method of Wassermann. As far as I can ascertain, Wassermann has made no important changes in his technic as described in his early papers and still uses salt solution extracts of syphilitic liver for antigen, although he cannot have failed to note the success of alcoholic extracts of normal tissues and, indeed, the general superiority of these extracts; apparently he continues to use the same hemolytic system and the same general technic described in 1906 and 1907. The term "Wassermann reaction," therefore, should, strictly speaking, be confined to a test conducted with Wassermann's original technic, but practically the latitude may be broader and embrace those modifications employing alcoholic extracts of luetic and non-luetic tissues as antigens, and such changes as using each constituent of the test in one-half, one-quarter, or one-tenth the amounts of the original Wassermann test, inasmuch as these changes do not alter the principles and are used mainly for economy.

However, all tests employing a different method for adjusting the hemolytic system, a method for titrating complement, a different hemolytic system, the use of active instead of heated serum, the utilization of natural complements and amboceptors in patients' sera, etc., cannot claim the designation "Wassermann test," being based upon principles and technic too remote from Wassermann's technic; modifications of this nature are now quite numerous and are usually designated as modifications of the Wassermann test.

The Demand for Standardization of Technic.—Probably no laboratory test has been subject to as much favorable and unfavorable criticism and to as many modifications in technic as Wassermann's serum test for syphilis; owing to the fact that the reaction is not strictly or biologically specific for syphilis and requires several biologic reagents of varying properties, it is readily subject to error in both a positive and negative way unless carefully and intelligently understood and conducted.

Many of the proposed modifications of the original technic have been demanded by an increasing experience and knowledge of the reaction, and several have undoubtedly served to improve its delicacy and value, but the employment of different methods by various persons has led to wide variation in the results, confusion in regard to the actual value of the test, and insistent demand for one standard method.

Within recent years several publications have drawn particular attention to discrepancies in the results of Wassermann reactions with the same

serum in different laboratories and, indeed, to varying results reported by the same serologist on specimens of blood from the same individual withdrawn at the same time and under identical conditions. For example, Uhle and MacKinney,¹ who submitted specimens of blood from each of 292 individuals to from four to ten laboratories, found that "there is one chance in five that the tests will agree"; Phelps² in a similar analysis of the results of Wassermann tests with 358 specimens of blood divided and sent to from two to four laboratories, found that the results agreed in but from 52 to 65 per cent. and Wolbarst,³ in a study of 37 cases by two or more serologists, found that the results agreed in 70 per cent. and disagreed either slightly or absolutely in 30 per cent. One of the most thorough studies of this kind has been more recently made by Palmer⁴ who submitted 75 serums to eight different laboratories with considerable variation in the reports. Palmer is of the opinion that a safe and efficient standardized technic should be adopted and required of all laboratories and that a higher degree of efficiency should be demanded of technicians.

I believe all serologists of experience agree that a certain percentage of discrepancies are to be expected with the use of different hemolytic systems and reagents and particularly different antigens, even granting that all tests were conducted with the requisite technical care and attention to details. All of the above-mentioned authors in addition to Pusey,⁵ Shropshire,⁶ and others have emphasized the need of a standardized technic; Brem,⁷ Faller,⁸ Schlesinger,⁹ and numerous others have directed attention to sources of error in the technic.

The Author's Investigations on Standardization of Technic.—Possibly the easiest way of standardizing the Wassermann test would be to adopt the original technic with a change in the antigen. But such a proposal would receive little recognition, not only because the original technic is defective in sensitiveness but also because the antigen is the greatest single source of contention and disagreement.

In my opinion "standardization" means a real, earnest, and unbiased study of different methods and of each and every phase of the complement-fixation test, for the purpose of determining by actual trial what is best and incorporating the facts into a test which will have for its purposes the establishment of a technic (1) of superlative sensitiveness; (2) of practical specificity; (3) of technical accuracy and uniformity of results; (4) yielding a true quantitative reaction; (5) as simple, and (6) as economical as possible.

Realizing my complex nature of the Wassermann reaction and the variable properties of its several biologic reagents, our almost complete ignorance of its mechanism and the absence of a specific and wholly satisfactory antigen, the task of even attempting standardization was considered a serious and laborious problem; knowing that the majority of serologists had an individual way of conducting certain steps in the technic and particularly that many had learned from experience to rely so firmly upon their own method as to be very loath to accept any other, the hope of building up a widely acceptable technic would appear almost hopeless unless an

¹ Jour. Amer. Med. Assoc., 1915, lxxv, 863.

² Boston Med. and Surg. Jour., 1915, clxxiii, 391.

³ New York Med. Jour., 1913, xcv, 378.

⁴ Jour. Amer. Med. Assoc., 1922, 79, 724.

⁵ Jour. Amer. Med. Assoc., 1913, lxi, 1920.

⁶ Southern Med. Jour., 1916, ix, 205.

⁷ Californ. State Jour. Med., 1912, x, 362.

⁸ Lancet-Clinic, 1914, cxii, 536.

⁹ Med. Rec., 1915, lxxvii, 61.

unexpected discovery bestowed upon the standard technic an indisputable quality of excellence. The method pursued in this investigation, which has covered a period of five years,¹ was to become acquainted with all existing methods by thoroughly reviewing the available literature, and by means of personal communications and interviews with a large group of serologists and *submitting the whole to careful unbiased experiment* and choosing that proving best on the basis of actual trial and receiving endorsement on the basis of experience.

This work has resulted in the building up of a new test² embracing a new antigen³ and many technical improvements. The antigen and technic of the test are given in this chapter, but owing to lack of space detailed accounts of the work conducted cannot be given. These results as well as an account of investigations in complement fixation in bacterial infections and for the differentiation of proteins, will be presented by the writer in a separate monograph.

GENERAL TECHNIC

In the preparation of glassware and biologic reagents employed in the Wassermann and other complement-fixation tests for syphilis, the general technic is the same and may be described collectively; the details covering amounts to employ, technic of titration, etc., are given in the descriptions of the various tests.

The Glassware.—*Test-tubes* should be of convenient sizes, perfectly clean, free from acids and alkalies, and preferably sterile. They need not be plugged with cotton, as it suffices to sterilize them in a wire basket with their mouth-ends downward. A method for cleaning test-tubes and other glassware is described on page 7.

Pipets should be perfectly clean and preferably sterile. Three kinds are required: The ordinary 1 c.c. pipet, graduated to 0.01 c.c. and calibrated to the tip; 5 and 10 c.c. pipets divided into 0.1 c.c. Care should be exercised in handling pipets to avoid breaking the tips. After use they should be washed free from blood, serum, etc. The handling and care of pipets are described on page 4. It is well to have a separate 1 c.c. pipet for each serum to be tested. If one pipet is employed for all sera, due care must be exercised in washing it out very thoroughly.

The *technic for preparing saline solution* is described on page 9.

The Serum and Cerebrospinal Fluid.—(a) **Serum.**—As a general rule all specimens of blood submitted for complement-fixation tests should be collected aseptically in sterile containers. This is especially necessary when there has been delay in transmitting the fluid to the laboratory, is when sent through the mails from distant points. When the reactions are to be conducted on the same or on the following day, the specimen of blood may be collected in chemically clean but not necessarily sterile containers. Bacterial contamination renders a fluid anticomplementary and unfit for complement-fixation tests. Specimens should be kept in a cold place until used.

Collecting Blood for Complement-fixation Tests.—In collecting blood for the Wassermann reaction the following points should be remembered:

1. That during active antisyphilitic treatment the blood may react negatively, whereas at a later period a true positive reaction is observed.

¹ See series of thirty-two papers published in the Amer. Jour. of Syph. commencing 1919, iii, 1 and ending 1922, vi, 680.

² Amer. Jour. Syph., 1922, vi, 82.

³ Amer. Jour. Syph., 1922, vi, 74.

It is well, therefore, not to collect blood until all specific treatment has been suspended for at least two weeks.

2. That blood collected during or immediately after an alcoholic debauch may yield a false negative reaction (Craig and Nichols).

3. That blood should not be collected just after anesthesia or while the patient has a high temperature.

As a general rule, at least 1 c.c. of serum and 2 c.c. of cerebrospinal fluid are required for making the syphilitic reaction. From 2 to 3 c.c. of blood are needed, these amounts being easily collected from adult persons by pricking the finger deeply and filling a small test-tube or vial, as shown on p. 15. This method is very convenient, especially for physicians, hospitals, and dispensaries where direct access to a laboratory can be had. When the treatment is to be guided by the Wassermann reaction, a number of tests are required, and patients may object to repeated venipuncture, whereas no objections will be raised to simple puncture of the finger.

Larger amounts of blood are collected from a vein at the elbow under aseptic precautions, as described on p. 18. As a rule, it is well to collect at least 5 c.c. of blood, especially if the specimen is shipped from a distant point (Fig. 129). An excess of serum permits the technician to repeat a test when necessary, or to apply more than one method, and thus at times both the physician and the patient are saved the time and annoyance incident to collecting another specimen.

The Keidel tube, which is sterilized and ready for use, is quite a convenience (p. 20). However, a test-tube or a centrifuge tube may be used, or, when a specimen is to be mailed, a 5 or 10 c.c. vial of thick glass, stoppered with a cork or a rubber stopper, is quite satisfactory (Fig. 108). Vial, stopper, and needle are readily sterilized in boiling water, drained, and cooled, the specimen collected, the vial tightly stoppered, and the whole sent at once to the laboratory. Cotton stoppers are unsatisfactory, as unless the tube or vial is maintained in an upright position, the fluid may be absorbed and rendered anticomplementary. *When specimens of blood are to be mailed, it is better to fill a small vial than to place the same amount in a large container, for in the latter case agitation through handling may result in so much mechanical hemolysis taking place as to render the serum unsatisfactory for use. Specimens so collected may be sent for long distances, even in warm weather, and undergo no change.*

In collecting blood from children, or where the veins are small, a proportionately smaller needle may be used. In infants the cupping apparatus of Blackfan is quite satisfactory (p. 22); frequently sufficient blood may be obtained from a large toe.

Preparing the Serum.—The specimen of blood should be kept in a cold place, and the serum removed at the end of twenty-four hours. Serum that is allowed to remain with the clot for longer periods is more likely to become anticomplementary, especially if it becomes deeply tinged with

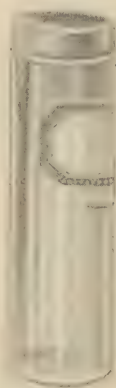


FIG. 129.—A VIAL TO CONTAIN BLOOD FOR THE WASSERMANN REACTION.

This is an ordinary glass vial fitted with a rubber stopper. It holds 5 c.c. to the mark, and is readily packed for mailing. Never stopper with cotton. A good cork stopper may be used.

hemoglobin. In cases where the serum does not separate, the clot may be broken up gently with a sterile glass rod and centrifugalized. The serum should be clear and free from corpuscles. Opalescent and milky serums, obtained during the period of digestion and from nursing women, usually do not interfere with the reaction; bile-stained serum may at times give marked non-specific fixation of complement.

Heating the Serum.—In the original Wassermann test the serum was heated to 56° C. for thirty minutes and in many modifications of the test this practice has been adopted. Some complement-fixation tests, however, employ unheated serum in order to increase the sensitiveness of the reactions by conserving all the syphilis "reagin."

Serum is heated for these purposes: (a) To remove thermolabile anti-complementary substances; (b) to remove the possibility of proteotropic or non-specific reactions, and (c) to inactivate the native or natural complements. Heating serum to 56° C. for thirty minutes results in the destruction of a portion of the syphilis "reagin"; unless a serum is strongly anticomplementary, I believe that *heating to 55° C. for fifteen minutes is sufficient* and results in much less destruction of "reagin."¹

In order to conduct a reliable test it is usually necessary to secure fresh serum. *This anticomplementary action of serums is so important that in every complement-fixation test there is a serum control tube containing all the ingredients except antigen, the object being to discover any inhibitory action of the serum itself upon the complement.*

Wechselmann's method of converting syphilitic serums showing a negative or weakly positive reaction to those exhibiting a marked positive reaction depends upon removing the excess of indifferent and inhibiting serum components and upon the destruction or diminution of the natural anti-sheep amboceptor present in so large a percentage of human serums. As Noguchi and Bronfenbrenner have pointed out, this method may likewise remove the antibodies concerned in the reaction. To 1 c.c. of heated serum add 3.5 c.c. of saline solution and 0.5 c.c. of a 7 per cent. suspension of freshly precipitated barium sulphate; shake, and let it stand for one hour at 37° C.; centrifugalize, and pipet off the diluted serum, which is now ready to be tested (1 c.c. = 0.2 c.c. of undiluted serum).

Cadaver serums are likely to be highly discolored with hemoglobin and quite anticomplementary. Such serums may be tested in half the usual dose, and while the results are quite specific, they are not so reliable or constant as those obtained from the living.

The *doses of serum* used in testing for the syphilis reaction are given with each method. In the original Wassermann test 0.2 c.c. was used. As a rule, from 0.05 to 0.2 c.c. of serum are satisfactory; higher doses may occasionally show a stronger positive reaction, but the serum must be perfectly fresh to avoid non-specific complement fixation.

(b) **Cerebrospinal Fluid.**—In certain nervous diseases the cerebrospinal fluid is examined for the syphilis reaction. *Fluid is secured* by lumbar puncture, according to the method described on p. 25. If the specimen contains blood, it should be centrifuged until it is clear. It should not be heated before use, as it does not contain hemolytic complement, and fresh fluids from cases other than syphilitics do not react positively. Cerebrospinal fluids, as a rule, possess weaker fixing powers than the corresponding blood-serum, and hence it is necessary to use larger doses—at least 0.5 to 1 c.c., instead of 0.05 to 0.2 c.c.—as in the case of blood-serum.

(c) **Other Fluids.**—Positive syphilitic reactions have been described as

¹ Amer. Jour. Syph., 1920, 4, 641.

occurring with milk, pleural and peritoneal exudates, and albuminous urine (Bauer and Hirsh) from luetic cases. The material should be perfectly fresh, as anticomplementary action is likely to occur and all require titration for this property in order to avoid non-specific reactions.

COMPLEMENT

While complement is to be found in the fresh normal serum of practically all warm-blooded animals, not all are suitable for complement-fixation tests. A suitable complement must possess two important properties: (1) Hemolytic activity, or the power of activating a hemolytic amboceptor, and (2) fixability, or the power of being "fixed" by antigen and antibody. Noguchi and Bronfenbrenner¹ have studied the complements of the dog, sheep, hog, ox, rabbit, and other animals, and found that the complement of the guinea-pig was best adapted, from all standpoints, for the complement-fixation test. The complements of pigs and sheep are quite fixable, but their weak hemolytic action and rapid deterioration render them unsuitable for fixation purposes. Rabbit complement is quite active, but is not easily fixable. Similar results have been observed by Matsunami, Trist, and myself.² Kolmer, Yui, and Tyau³ found rat complement fairly well suited for making the syphilitic reaction with an antihuman hemolytic system.

The hemolytic power of guinea-pig complement is not constant. In unhealthy animals it is likely to be low, and even among normal animals it may show some variation. For this reason the hemolytic power of each serum is determined by a method of titration before complement-fixation reactions are conducted. Fixed doses of hemolysin and corpuscles may be used, and the amount of complement necessary for effecting complete hemolysis may be determined, or a fixed dose of complement and corpuscles may be used with different amounts of hemolysin, the chief object being to adjust all three factors of the hemolytic system, namely, complement, corpuscles, and hemolysin, to exact and known proportions.

The complement in the serums of different guinea-pigs may show considerable variation in fixability. The amount of complement inhibited by serum alone and organic extract alone, or by given constant quantities of serum and extract, may vary more markedly than their complementary activity. To reduce this error to a minimum it is advisable, whenever possible, to use the pooled serums of two or more pigs for making complement-fixation tests.

Preparation of Guinea-pig Complement Serum.—The animal is bled under ether anesthesia into a Petri dish or centrifuge tube. The large vessels on both sides of the neck are quickly severed with a pair of sharp-pointed scissors or a scalpel, care being exercised not to sever the trachea and esophagus. A funnel is used for collecting blood in centrifuge tubes. It is well finally to sever the spinal cord in order that the animal may not recover from the anesthetic and thus insure a painless operation throughout (see p. 37).

The following technic is employed by the author⁴:

1. Select large well-nourished guinea-pigs which have not been fed within twelve to twenty-four hours of the time for bleeding; avoid pregnant animals.

2. Collect blood in a *chemically clean* and preferably sterile centrifuge tube by means of a clean funnel about 4 inches in diameter, or in a Petri

¹ Jour. Exper. Med., 1911, 13, 78.

² Amer. Jour. Syph., 1919, 3, 407.

³ Jour. Med. Research, 1913, xxviii, 483.

⁴ Amer. Jour. Syph., 1919, 3, 407.

dish. We prefer the former because if separation of serum is unsatisfactory the clot may be gently broken up with a glass rod and the serum secured by centrifuging the material. Chemical cleanliness is essential because traces of acids or alkalies are destructive of complement.

3. If guinea-pigs are to be bled to death, stunning the animals with a blow at the base of the skull is preferable to ether and chloroform anesthesia because cardiac activity is not interfered with and the maximum amount of blood is obtained. Guinea-pigs may also be bled from the ears without anesthesia, or from the heart (p. 37) or an external jugular vein under light ether anesthesia.

4. In bleeding a guinea-pig to death it is not necessary to shave the neck; a few hairs in the blood do not alter the properties of the serum. The great vessels on one or both sides should be *quickly* severed and preferably with a pair of stout sharp scissors with one pointed blade to facilitate piercing of the skin and underlying tissues. It is easy to avoid cutting the trachea and esophagus, but it does not appear to make any difference whether the trachea is cut; the esophagus should not be cut, because in squeezing the abdomen to pump out blood in the great vessels stomach contents may escape, although even this accident is rare in our experience.

5. In bleeding from the heart a chemically clean and sterilized 5 to 10 c.c. all glass or glass-metal syringe fitted with a short needle of about gage 20 may be used; in bleeding from an external jugular vein the hairs are plucked just above the region of the clavicle and a small incision made through the skin and vein, with collection of blood by means of a funnel into centrifuge tubes. As soon as the animal is released bleeding ceases and the wound heals promptly and without infection.

6. *Do not use the blood immediately after bleeding*; if the animals are bled on the day the serum is to be used, place the clots in the incubator at 37° C. for an hour, followed by breaking up each with a glass rod and centrifuging, or let the clots stand at room temperature for two or three hours for separation of serum which if not complete may be finished by centrifuging. Animals may be bled late in the afternoon of the day before and the clots placed in the incubator for one hour or left at room temperature for two hours and then placed in a refrigerator until the following day. If the sera have separated poorly, gently break up the clots and centrifuge. *It is preferable to leave the serum on the clot in the refrigerator until ready for use; unused serum should be returned to the refrigerator until required.* Traces of hemoglobin in the serum do not interfere, but the serum should be free of corpuscles.

Moore¹ and, more recently, Ecker² have described guinea-pigs naturally deficient in complement. According to Moore, this property is inheritable and apparently is not due to anything interfering with the action of hemolysin.

As a general rule, therefore, it is good practice to bleed the animal late in the afternoon preceding the day on which the experiment is to be made, or at least some hours before the regular work of the day begins. The serum should be clear and contain no corpuscles.

Preservation of Complement.—Various methods have been advocated from time to time for the preservation of complement serum. These have been subjected to a comparative study by Kolmer, Matsunami, and Trist,³ and the following are to be recommended:

1. *In a Frozen State.*—Serum is placed in ampules in amounts of 1 c.c.,

¹ Jour. Immunology, 1914, 4, 425.

² Jour. Infect. Dis., 1921, 29, 611.

³ Amer. Jour. Syph., 1919, 3, 513.

sealed, and kept in a refrigerator at or near 0° C. or in mixtures of ice and salt. Vacuum bottles have been advocated. A home-made "Frigo" is easily constructed as described on page 56.

2. *With Sodium Chlorid (Author's Method).*—To each cubic centimeter of serum add 0.2 gm. chemically pure sodium chlorid, dissolve, and keep at a low temperature. When to be used dilute each cubic centimeter with 19 c.c. of distilled water which gives a 1 : 20 dilution in 1 per cent. saline. This method is particularly useful when complement is employed in a 1 : 20 dilution.

3. *With Sodium Chlorid After the Method of Thompson.*¹—An 8.1 per cent. solution of pure sodium chlorid in distilled water is prepared and autoclaved; equal parts of complement serum and this salt solution are prepared, placed in ampules, and kept at a low temperature. When required, 1 c.c. of the mixture is diluted with 4 c.c. of distilled water which gives a 1 : 10 dilution of complement in isotonic solution. Dilutions of 1 : 20 or 1 : 30 may be made from this 1 : 10 solution.

4. *With Sodium Chlorid After the Method of Austin.*²—A 25 per cent. solution of pure sodium chlorid in distilled water is prepared and sterilized; to 1 part of complement serum is added 1½ parts of this solution and the mixture stored in ampules at a low temperature. When required 1 c.c. is diluted with 3 c.c. of 0.5 per cent. salt solution which gives a 1 : 10 dilution of complement in an isotonic solution.

5. *With Sodium Chlorid After the Method of Neill.*³—A saturated solution (approximately 36 per cent.) of pure sodium chlorid is prepared in hot distilled water, cooled, filtered, and sterilized; to each cubic centimeter of complement serum is added 0.1 c.c. of this salt solution and the mixture kept at a low temperature in ampules. When required 1 c.c. is diluted with 3 c.c. of distilled water and further diluted with 6 c.c. of physiologic saline solution which gives a 1 : 10 dilution.

6. *With Sodium Acetate After the Method of Rhamy.*⁴—A 10 per cent. solution of pure sodium acetate in 0.9 per cent. solution of sodium chlorid is prepared and sterilized; to 1 part of complement serum is added 1½ parts of acetate solution, and the mixture kept at a low temperature in ampules. When required 1 c.c. is diluted with 3 c.c. of saline solution which yields a 1 : 10 dilution. Higher dilutions may be prepared as desired.

Complement serum preserved by these methods will usually maintain its hemolytic and fixing properties for several weeks; *loss in fixability or delicacy in the complement-fixation test is usually apparent before an appreciable deterioration in hemolytic activity.*

The Amount of Complement to Employ.—And now we come to a very important question, namely, the amount of complement that is to be used in conducting complement-fixation tests. The hemolytic system should be so adjusted that the amount of complement employed is neither unnecessarily large, which tends to falsely negative reactions in the presence of small amounts of antibody, nor too small, which does not allow for the anti-complementary activities of serum alone and antigen alone and for complement destruction during the primary incubation. For practical purposes in routine complement-fixation tests when the complement is titrated plain (that is, in the absence of antigen and normal serum) it is necessary to use at least 2 units of complement and 2 units of hemolysin to allow for anti-

¹ Jour. Amer. Med. Assoc., 1916, lxvi, 652.

² Jour. Amer. Med. Assoc., 1913, lxii, 868.

³ Public Health Reports, August 23, 1918, 1387.

⁴ Amer. Med. Assoc., 1917, lxi, 973.

complementary action of serum and antigen and destruction of complement, as shown in the comparative studies of Kolmer, Matsunami, and Rule.¹

Some present-day observers use exactly one unit of complement and one unit of hemolysin. This is permissible, providing the complement is titrated in the presence of a constant dose of antigen and a constant dose of serum, in order that due allowance for the anticomplementary action of these may be made in the titration. Under these circumstances, however, it is necessary to titrate each patient's serum with the complement, because one serum or even the pooled serums of different persons should not be taken as a standard in the titration, for two important reasons: (1) The patient's serum which we are about to test may be more anticomplementary than the serum used in the complement titration, and hence when used in the main test, with exactly one unit of complement, mild degrees of inhibition of hemolysis will be secured that may be interpreted as slightly positive reactions; or (2) the serum used in the complement titration may contain more or less natural hemolytic amboceptor than the patient's serum, and this factor exerts an influence on the titration, so that the unit varies with different serums. For these reasons I have included here a fourth method for using a single unit of complement under conditions where the anticomplementary action of *each* serum and the *antigen* are determined, in preference to titrating the complement with a serum and using this unit for a number of other serums that are sure to differ from each other.

In complement titration, therefore, we determine the amount or unit of complement necessary to produce hemolysis with fixed amounts of hemolysin and corpuscles. It will be observed that I use two units of hemolysin, as determined by a previous titration. These two units are equivalent to *one dose*, and the same would be true whether three, four, five, or more units were used, because in this titration the corpuscles and hemolysin are arbitrary and fixed constants, and are used for determining the amount of complement necessary to bring about complete hemolysis.

In conducting the main tests the dose of corpuscles and that of hemolysin are the same as those used in the complement titration, but instead of using exactly one unit of complement it is necessary to use two units, as stated above, to allow for the anticomplementary action of antigen and patient's serum.

It is important to remember that, in conducting this titration, hemolysin, complement, and corpuscles are to be mixed at once; if hemolysin and corpuscles are mixed and allowed to stand for ten minutes or more before receiving the complement the corpuscles become "sensitized," and the amount of complement required for effecting hemolysis will be less than if all three are mixed one after another. If this rule is not adhered to an error in technic may result.

HEMOLYSIN

Since the original work of Wassermann appeared the antisheep hemolytic system has been most widely used in complement-fixation tests.

Antisheep hemolysin is readily prepared by immunizing rabbits with washed sheep's corpuscles. A simple and efficient method is to give intravenous injections of five doses of 5 c.c. each of a 10 per cent. suspension every three or four days. Other methods and the details of the preparation and preservation of hemolysin are given in previous chapters.

Objections have been made to the use of the antisheep hemolytic system

¹ Amer. Jour. Syph., 1920, 4, 518.

because of the presence, in a large proportion of human serums, of variable amounts of natural hemolysin for sheep's cells. In about 90 per cent. of fresh inactivated human serums sufficient hemolysin is present partially or completely to hemolyze the usual dose of sheep-cell emulsion with the customary amount of guinea-pig complement. In fact, the Bauer and Hecht modifications of the Wassermann reaction utilize this natural hemolysin, but, as will be pointed out further on, this factor is too variable to be employed in conducting the reaction, as non-specific or false positive results are quite likely to occur.

As has been stated in the preceding chapter, the delicacy and accuracy of any complement-fixation test depend to a large extent upon proper adjustment of the hemolytic system. It will readily be understood that the presence of an unknown quantity of natural hemolysin in a serum is a drawback to accurate quantitative estimations. The importance of this lies in the fact that for some unknown reason an excess of hemolysin may completely hemolyze the corpuscles, even though a small amount of the necessary complement has been specifically fixed by antigen and syphilis antibody. In this manner negative reactions may result with serums that would otherwise show a slight positive reaction. To remove this source of error Noguchi has advocated the use of an antihuman hemolytic system, which renders the reaction more delicate. However, comparative studies between antisheep and other hemolytic systems by Kolmer, Matsunami, and Rule¹ demonstrate that, with proper technic, the influence of natural hemolysins may be reduced to a minimum and rendered almost negligible.² At any rate it is very simple if desired, and but little trouble to remove the antisheep hemolysin routinely from human serums previous to making the tests (for technic, see p. 399). Furthermore, the influence of natural hemolysin may be prevented by *heating the tubes in a water-bath to 55° C. for ten minutes* after the secondary incubation, as I have recently described.³ This inactivates complement and thereby breaks up the hemolytic system.

Methods for titrating immune hemolysin will be dealt with in giving a detailed description of the various methods that follow.

RED BLOOD-CORPUSCLES

Defibrinated sheep blood is washed three times with an excess of sterile normal salt solution to remove all traces of serum. Methods for collecting, washing, and preserving blood-cells are described on pages 11-13.

In the original Wassermann reaction a 5 per cent. suspension in salt solution is used in doses of 1 c.c. This emulsion is quite heavy, and sharper and clear results are secured by using just half this amount and at the same time sufficient cells are used to make the readings easy and distinct. Either 0.5 c.c. of a 5 per cent. or 1 c.c. of a 2.5 per cent. suspension may be used. I have used the latter with entire satisfaction for several years.

The suspension of cells is prepared with sterile 0.85 per cent. sodium chlorid solution. To 2.5 c.c. of corpuscles sufficient salt solution is added to bring the total volume of the emulsion up to 100 c.c., or smaller amounts may be prepared by suspending 1 c.c. of corpuscles in 39 c.c. of salt solution.

Before each day's work the amount of corpuscle suspension needed should be calculated, and sufficient for the day prepared at one time, for if a fresh suspension is prepared later, titration with the complement and hemolysin would be required. Attempts to count the corpuscles in suspension can only be regarded as approximate and are unreliable. *By titrat-*

¹ Amer. Jour. Syph., 1920, 4, 278.

² Ibid., 1920, 4, 135.

³ Ibid., 1921, 5, 628.

ing each suspension with the complement and hemolysin to be used, that particular emulsion is thereby adjusted, so that it is immaterial whether a few more or a few less corpuscles are present.

Sheep's blood is obtained either from an abattoir or by bleeding an animal from the external jugular vein (p. 38). The latter method is preferable, but due care must be exercised not to bleed too frequently or in excessive amounts, as if anemia occurs the corpuscles become unduly fragile.

Sheep's cells are easily preserved in a satisfactory condition for forty-eight hours by first washing them and then storing the sedimented corpuscles in a good ice-chest. Suspensions are less easily preserved. It is best to use fresh corpuscles, and those that are several days old and unduly fragile should never be used.

The following technic for preparing suspensions may be employed as recommended by Kolmer and Brown¹:

1. A satisfactory electric or water centrifuge should be available for washing the blood; the former is to be preferred. The instrument should be started and stopped slowly.

2. Washing the blood is best accomplished in accurately graduated centrifuge tubes. Those tubes may be sterilized prior to use, but this is not necessary; they should be chemically clean and rinsed with physiologic saline solution before use.

3. For washing blood physiologic saline is employed (0.85 per cent. sodium chlorid in distilled water).

4. In washing defibrinated blood one volume of blood is placed in a centrifuge tube, two volumes of saline solution added and gently mixed; citrated blood collected in the proportion of 1 part blood to 4 parts citrate solution is used without further dilution. Each tube must be accurately counterbalanced in the centrifuge and whirled until all corpuscles have been thrown down, the time required depending upon the speed of the centrifuge (first washing).

5. The supernatant fluid is carefully removed down to the corpuscle mass with a capillary pipet (attached to suction pump or rubber teat), and at least three to five volumes of saline solution added; by capping the tube and inverting all the corpuscles are thoroughly but gently stirred and mixed in the saline solution, and the tubes again centrifuged (second washing).

6. The supernatant fluid is again removed, replaced with saline solution, the corpuscles thoroughly mixed and again centrifuged for *twice as long* as found necessary to throw down the corpuscles in the second washing. This longer period of centrifuging is advisable to firmly and evenly pack the washed cells and *the speed and duration of centrifuging should be uniform in each laboratory as based upon experience with the particular centrifuge in use.*

7. If the supernatant fluid is discolored with hemoglobin the cells should be washed again until the supernatant fluid is practically colorless.

8. With the last washing the centrifuge is stopped slowly so as not to disturb the corpuscle mass, and *the volume of corpuscles read in the centrifuge tube before removing the supernatant fluid*; the latter is now removed and a suspension of proper strength prepared. A 5 per cent. suspension, for example, is prepared by washing the cells from the centrifuge tube into a proper flask with nineteen times as much saline solution as corpuscle mass.

9. As the corpuscles in suspension tend to settle rapidly, the suspension should be *gently and thoroughly shaken* by hand before being used and *dur-*

¹ Amer. Jour. Syph., 1919, 3, 169.

ing the time of use, if several minutes are required for pipetting the suspension; otherwise the doses will be uneven and inaccurate.

10. So far as practicable the suspension should be kept in a refrigerator when not being used.

ANTIGENS

As was previously stated, the ordinary alcoholic extracts of syphilitic livers used as "antigens" in conducting the Wassermann reaction are not biologically specific. It is generally accepted that even in watery extracts of syphilitic livers the main antigenic principles are lipoidal substances, independent of the *Treponema pallidum* itself. Next to pure cultures of pallida, these aqueous extracts of luetic livers come closest to being a specific biologic antigen. Although alcoholic extracts of luetic liver may contain special lipoidal substances that enhance their efficacy as antigens, yet, as shown by Noguchi, and as confirmed later by us, alcohol does not serve well to extract pure cultures of pallida, and therefore these extracts can hardly be regarded as specific, in the sense that they contain antigenic principles of the spirochetes themselves. The only specific biologic antigen is an aqueous extract of a pure culture of pallida; this antigen is, however, much less serviceable than an ordinary organic extract because the Wassermann reaction depends upon the peculiar lipodophilic "reagin," which absorbs complement with lipoids in a characteristic but biologically non-specific manner.

The term "antigen," as ordinarily used in the Wassermann reaction, must therefore be regarded as a misnomer. It is, however, so generally used that it may be retained with a distinct understanding as to its actual meaning.

With the discovery that alcoholic extracts of normal organs may serve as antigen and that the chief antigenic principles reside in the lipoids, it followed that extensive researches were undertaken in the hope of discovering a lipid, or a combination of lipoids, that would prove sufficiently delicate to act specifically in the serum diagnosis of syphilis, and not with normal serums or those of persons suffering from other diseases. As a result a large number of different extracts are in use. Each has its own advocates, so that the general subject of antigens is the most complicated one with which we have to deal in performing the Wassermann reaction.

While various organic extracts may be used, practical experience has shown that some are better than others. It is important to remember:

1. *That all antigens are capable in themselves of absorbing a certain amount of complement.* This is due to the presence of undesirable extractives, some preparations containing more than others. In certain doses, however, all antigens are capable of exerting this *anticomplementary action*, and, other things being equal, that antigen is best which shows this non-specification in the smallest degree. Before an antigen may be used in conducting any complement-fixation test it is necessary to ascertain its anticomplementary dose, for if this dose were used, a portion of or all the complement would be fixed in a non-specific manner, so that hemolysis, being partial or absent, yields false positive reactions.

2. *Most antigens, when in sufficiently large amounts, are hemolytic, i. e., they may hemolyze corpuscles in a non-specific manner.* This hemolytic action is usually due to the presence of undesirable extractives, and extracts of organs that have undergone advanced autolysis or fatty degeneration are known to contain more of these hemolytic substances than do extracts of normal organs. As a general rule, a highly anticomplementary

antigen is likely to be correspondingly highly hemolytic. The hemolysis may be due to the presence of lipoidal substances or to the alcohol used in preparing the extract. If an antigen were used in an amount equal to its hemolytic dose, partial or complete hemolysis would occur in all tubes, so that a false negative result would be secured. As a rule, the hemolytic dose of an antigen as determined by titration in the presence of serum is larger than the anticomplementary dose, so that if the latter is known, it is not always necessary to determine the former.

3. *Practically every alcoholic organic extract will serve, in certain amounts, to absorb complement in the presence of the serum of a syphilitic person.* Some extracts, however, will do this better than others. The Wassermann reaction depends upon the fact that a larger amount of complement is fixed by the syphilis antibody and extract than is fixed by normal serum or the serum of a person with some disease other than syphilis and this same extract. The only notable exception to this general rule is to be found with the serum of frambesia. The amount of antigen that is found, by a process of titration, to fix a large amount of complement with a constant dose of syphilitic serum is known as its *antigenic unit*. Not every lipid serves equally well as antigen, and therefore considerable research work has been done in the hope of discovering an extract or a combination of lipoidal substances that would show a constant reaction and would react only with the syphilis antibody. Thus far this has not been accomplished; unfortunately, pure pallida antigens are not entirely specific or serviceable, and if the specific and ideal antigen is discovered in the future it will probably be of the nature of a lipoidal substance, altered or produced in a specific manner by the *Treponema pallidum* itself. In the meantime we have antigens sufficiently delicate and specific, when properly used, to render the Wassermann reaction of great value in the diagnosis of syphilis and to serve as a guide to its treatment.

From a practical standpoint, therefore, to be suitable for use as antigen in the syphilitic reaction any extract or preparation must fulfil the following requirements:

1. It should be largely free from anticomplementary action.
2. It should likewise be free from hemolytic action, in small doses at least.
3. It should possess a high degree of sensitiveness for the syphilitic antibody, *i. e.*, be capable of absorbing relatively large amounts of complement in the presence of syphilitic serum. A good antigen is one that, in small amounts, is perfectly antigenic, and that does not become anticomplementary or hemolytic until from four to ten times this amount is used.
4. It should be quite stable and not difficult to prepare, and different preparations should bear a certain relationship to one another in their properties—that is, they should keep well, and different extracts prepared in the same manner should show fairly constant antigenic, anticomplementary, and hemolytic doses.

Preparation of Antigens.—The following antigens have been most widely used and recommended:

1. Aqueous extracts of syphilitic livers.
2. Alcoholic extracts of syphilitic livers.
3. Alcoholic extracts of normal organs.
4. Alcoholic extracts of normal organs reinforced with cholesterin.
5. Acetone-insoluble lipoids.
6. Alcoholic extracts of heart reinforced with lecithin and cholesterin (author's new antigen).
7. Aqueous extracts of pallidum culture.

1. Aqueous Extracts of Syphilitic Livers.—This is the original antigen, as employed by Wassermann, Neisser, and Bruck; Wassermann still uses these extracts in preference to others. They may contain spirochetes or their direct derivatives, and, as shown originally by Wassermann and Neisser, may be true biologic antigens, for when injected into monkeys antibodies are formed.

No satisfactory analyses of these extracts have been made. Chemically they differ in no essential respect from the liver of acute yellow atrophy (Ehrmann and Stern; Seligman and Pinkus). As antigen they are more efficient than similar extracts of normal liver. The nature of the specific factor has not yet been demonstrated with certainty. They react with the serums of yaws, and, as in the case of other antigens, their main antigenic principle is apparently due to the presence of lipoids.

They are less stable than alcoholic extracts, and are likely to become highly anticomplementary and lose their power of reacting with syphilitic serums. Citron is convinced that these changes are brought about by careless handling of the extract or its exposure to the light. He recommends that the extract be kept constantly in the ice-chest, and that it be kept out only long enough to remove sufficient for the day's work.

Preparation.—The fresh liver taken from a syphilitic fetus, and showing the presence of spirochetes by dark-ground illumination, is weighed and cut into fine pieces. Four times its weight of 0.5 per cent. phenol in physiologic salt solution is added. The mixture is placed in a brown bottle and shaken mechanically at room temperature for twenty-four hours. It is then filtered through gauze, to remove the larger particles, and stored in a brown bottle in an ice-chest. After several days of sedimentation the fluid assumes a yellowish-brown opalescence and is ready for the preliminary titration to determine its anticomplementary and hemolytic doses. The sediment should not be disturbed, but the supernatant fluid should be carefully removed by means of a pipet. According to Citron, extracts that must be used in quantities of less than 0.1 c.c. are, as a general rule, unsatisfactory. Only such extracts should be used as in doses of 0.4 c.c. will not interfere with hemolysis. The method of making these titrations is given later.

2. Alcoholic Extracts of Syphilitic Livers.—These antigens are extensively used. They are not true biologic antigens, for they do not give rise to antibodies (Schatilof and Isabolinsky; Seligman and Pinkus); they are, however, usually better antigens than similar extracts of normal liver, a fact that may be explained, in part at least, by chemical changes, namely, fatty changes, autolysis, soaps (Beueker), excess of cholesterin (Piglini), etc., which, while not specifically syphilitic in nature, are often produced to a striking degree in congenital syphilis.

Preparation.—Fetal liver known to contain numerous spirochetes is used in preparing this extract. Fresh organs may be examined at once by dark-field illumination, or if this is impossible and the fetus shows signs of syphilis, the liver may be cut into large pieces and preserved in 70 per cent. alcohol. After a few days a section is removed and stained by the Levaditi method for spirochetes. If these micro-organisms are numerous, the liver is suitable for preparing the antigen; otherwise it should be discarded. Very fatty livers are to be avoided, and those of stillborn fetuses are to be preferred.

Ten grams of liver are minced, ground with quartz sand, and treated with 100 c.c. of absolute ethyl alcohol. The mixture is shaken mechanically with glass beads for twenty-four hours, and extracted in the incubator for ten days. The containing flask or bottle should be well stoppered to prevent undue evaporation, and should be shaken up at least once a day. The extract is then filtered through fat-free paper or paper washed with ether and alcohol to remove the hemolytic substances that may be present. The filtrate is measured, and the loss by evaporation is made up by the addition of more alcohol. If a shaking apparatus is not at hand, extractions may be left in the incubator a few days longer. After standing a few days a sediment forms, which should not be removed or disturbed.

3. Alcoholic Extracts of Normal Organs.—These are used extensively at present, and apparently yield results equal to those obtained with ex-

tracts of luetic liver. It is certainly true that a good extract of a normal organ is superior to a poor one prepared from luetic liver. Many, with the idea of specificity uppermost in their mind, adhere to the use of the latter, whereas the results of research and of practical work shows that lipoids from normal organs serve equally well as antigen in making the syphilitic reaction, and, indeed, may prove superior if luetic liver is used that has undergone advanced fatty changes or autolysis, when undesirable hemolytic and anticomplementary derivatives are extracted in excess.

Human, guinea-pig, and beef-heart muscle are usually employed. The first is especially efficient and is to be preferred.

Preparation.—The organ is obtained fresh from the autopsy room. It is freed from fat, and to each 10 grams of minced muscle 100 c.c. of absolute ethyl alcohol are added. Extraction is conducted in exactly the same manner as was described in the preparation of alcoholic extract of luetic liver.

If guinea-pig heart is employed, as much of the fat as possible should be removed, otherwise the extract may be quite anticomplementary.

Boas prepared an extract of human heart by treating the ground muscle with nine parts of absolute alcohol, shaking for an hour at room temperature, filtering, and storing away in a stoppered bottle. He found that different extracts so prepared are remarkably constant in their properties, although they deteriorate rapidly and should be prepared freshly every few weeks.

4. Alcoholic Extracts of Normal Organs Reinforced with Cholesterin.

—Sachs¹ advocated the addition of pure cholesterin to alcoholic extracts of normal heart as a means of rendering these antigens more delicate without materially increasing their anticomplementary and hemolytic properties. He found that such preparations possess properties equal to the best syphilitic extracts. This work has been confirmed by McIntosh and Fieldes,² Walker, and Swift,³ Laubaug, Williams, Casselman, and myself⁴ and others. We have studied the subject with much interest, comparing the results with those secured from other antigens, as alcoholic extracts of syphilitic liver and acetone-insoluble lipoids. It is true that these preparations are highly sensitive—so much so that I never employ them alone in making diagnostic reactions, but always in conjunction with other extracts as controls, in order to detect and avoid non-specific reactions with non-luetic serums. We have found that they occasionally give faint positive reactions with normal serums; on the other hand, not infrequently, they react strongly positive in cases where, with other extracts, the reactions are negative; in the majority of such cases the serum is from a long-standing or a treated case of lues that needs further treatment until the reaction with a cholesterin extract becomes negative. These alcoholic extracts of normal organs have their greatest value, therefore, with known syphilitic serums when the reaction is conducted as a guide to treatment. In diagnostic reactions, however, it is my opinion that they should not be used alone, but together with less sensitive antigens. *In other words, one should use every precaution and exercise great care before making a diagnosis; when lues is known to be present, however, the treatment should be thorough, and there would seem to be no better criterion for judging the state of the infection than repeated negative reactions with cholesterin extracts.*

Preparation.—These extracts are prepared of human, ox, and guinea-pig heart. Human heart usually yields the best extract. Care should be taken to use only muscle and to avoid fat. To 10 grams of minced muscle add 100 c.c. of absolute ethyl alcohol. Shake

¹ Berl. klin. Wchn., 1911, 48, 2066.

² Ztschr. f. Chemotherapie, 1912, 1, 76.

³ Jour. Exper. Med., 1913, 18, 75.

⁴ Archiv. Int. Med., 1914, 12, 660.

in a mechanical shaker for twenty-four hours, and continue the extraction in the incubator for ten days or two weeks. Then filter through fat-free filter-paper, and add absolute alcohol to make up for the loss through evaporation. Add 0.2 gram of Kahlbaum's cholesterin (0.2 per cent.); shake well and stand aside in the refrigerator for a few days. The cholesterin goes into solution slowly in cold alcohol. After a week the extract may be again filtered and stored in a tightly stoppered bottle. The slight sediment that may form should not be disturbed.

These extracts keep fairly well. Different preparations are quite similar in their properties; they are usually found to be highly antigenic and no more anticomplementary than crude alcoholic extracts. They constitute, therefore, inexpensive and very sensitive antigens.

5. Acetone-insoluble Lipids.—As previously stated, crude alcoholic extracts may contain an excess of undesirable constituents, such as neutral fats, fatty acids, soaps, and certain protein materials, which are responsible for the untoward anticomplementary and hemolytic effects. To eliminate these Noguchi and Bronfenbrenner¹ advised the exclusive use of the acetone-insoluble fraction instead of the entire unfractionated alcoholic extract, especially if unheated human serums are used in conducting the syphilis reaction. These extracts are composed essentially of lecithins, which, when prepared from any one source, consist of a mixture of analogous bodies; lecithins from different sources vary in their composition. In speaking of lecithins, one is prone to regard them as chemicals, and to overlook their biologic properties. Noguchi no longer employs the term "lecithin" to designate the acetone-insoluble fraction of tissue lipoids.

These antigens are readily prepared of ox heart or of human liver, the former being preferable for use. Their main disadvantage is the expense of preparation, for it may be necessary to prepare several extracts before one that is satisfactory is secured. A good extract will, however, keep well, and is a reliable and valuable antigen for the testing for the syphilitic reaction.

Preparation.—A mashed paste of the muscle of ox heart is extracted with 10 parts of absolute alcohol at 37° C. for four days. It is then filtered through filter-paper and the filtrate collected and brought to a state of dryness by evaporation. The use of the electric fan is not necessary, for if poured into large flat dishes, the filtrate will evaporate in from twelve to twenty-four hours. The residue is then taken up with a sufficient quantity of ether, and the turbid ethereal solution is allowed to stand for a few hours in a cool place until cleared. The clear ethereal portion is then carefully decanted off into another clean evaporating dish, and then allowed to become concentrated by evaporating the ether off. The concentrated ethereal solution is now mixed with about 10 volumes of pure acetone. A light yellow precipitate forms, which is allowed to settle, and the supernatant fluid is decanted off. Dissolve each 0.3 gm. of this substance in 1 c.c. of ether and add 9 c.c. of pure methyl alcohol. As a rule, the greater part of the substance goes into solution. This alcoholic solution remains unaltered for a long time, and is kept as a stock solution from which the emulsion for immediate use may be prepared at any time by mixing 1 c.c. with 19 c.c. of saline solution. This solution is then titrated for antigenic, anticomplementary, and hemolytic action.

According to Noguchi, if the extract is anticomplementary or hemolytic in doses of 0.4 c.c. of a 1 : 10 dilution, it is unsuitable. If it produces complete inhibition of hemolysis with 0.1 c.c. of syphilitic serum in doses of 0.02 c.c. or less of the same dilution (= 0.2 c.c. of a 1 : 100 dilution), it is suitable. In making the fixation test, 0.1 c.c. of a 1 : 10 emulsion is to be used, thus employing five times the minimal antigenic dose which does not cause non-specific fixation and is not unduly sensitive.

6. A Cholesterolized and Lecithinized Alcoholic Extract of Heart Muscle (Author's New Antigen).—As a result of investigations upon the subject

¹ Jour. Exper. Med., 1911, 13, 43.

of antigens for the Wassermann test¹ a new method has been found very satisfactory. Due to the investigations of Noguchi,² Browning, Cruickshank and McKenzie,³ Erlandsen,⁴ Neymann and Gager,⁵ and others we now know that the lecithins (diaminomonophosphatids) are highly antigenic and very slightly hemolytic and anticomplementary, and Noguchi's method for extracting these from tissues (acetone-insoluble lipoids) is highly efficient and somewhat preferable to the more complicated procedure of Browning, Cruickshank and McKenzie. As shown by Erlandsen and Neymann and Gager, ether removes from tissues a large portion of the hemolytic substances, principally soaps, neutral fats, and fatty acids; also bile salts in extracts of liver tissue. Alcohol also removes some of these in addition to substances possessing marked anticomplementary activities, as proteins and protein cleavage products, cholesterol and other lipoidal bodies, and various salts. No doubt some of the latter are also antigenic, but at least the best of them, namely, cholesterol, can be prepared in a high state of purity and added to any extract for the advantage of its influence upon antigenic activity.

The following method utilizes these observations and the results of previous studies on antigens,⁶ and yields extracts of remarkable antigenic sensitiveness and slight anticomplementary and hemolytic activity; the principles of the method are as follows:

1. Dried human or beef-heart muscle is employed; there is no choice between the two tissues if both are *perfectly fresh*. Human heart muscle, however, is frequently fatty or may have undergone more or less postmortem change; if so, fresh lean beef-heart muscle is to be preferred. Dried tissue is employed for the following reasons: (a) It permits of the use of very finely divided tissue; (b) tissues are easily kept for long periods of time; (c) three or more heart powders may be kept, permitting of the use of mixtures of tissues and polytropic extracts which are probably better than preparing an extract of a single heart muscle.

2. The dried tissue is first extracted with ether, which removes a large portion of the hemolytic substances; the phosphatids (lecithins), which are also partly removed by the ether, are recovered by precipitation with acetone and returned to the extract.

3. The tissue is now dried and extracted with alcohol, which removes some antigenic and hemolytic substances and considerable anticomplementary substances. This alcohol is evaporated, extracted with ether, and precipitated with acetone, which removes the highly antigenic fraction of lecithins (phosphatids), which are later returned to the extract.

4. The tissue is now extracted for a second time with absolute ethyl alcohol which removes some antigenic principles and slight or almost negligible amounts of anticomplementary and hemolytic substances. The acetone-insoluble lipoids (lecithins, phosphatids) recovered from the preliminary ether and alcohol extractions are dissolved in ether and returned to this alcoholic extract with 0.2 per cent. cholesterol; the resulting extract possesses high antigenic activity, slight anticomplementary, and no hemolytic activity. The details of preparation of this extract are as follows:

Preparation.—(a) Fresh beef or human hearts are washed free of blood, dissected free of fat and large blood-vessels, and the muscle passed through a meat grinder three or four times. The minced tissue is then rapidly dried in a vacuum apparatus or equally well by spreading in *thin layers* on clean glass plates and drying by *rapid fanning*, preferably in a dust-

¹ Amer. Jour. Syph., 1922, 6, 74.

² Jour. Exper. Med., 1909, 11, 84.

³ Jour. Path. and Bacteriol., 1910, 14, 484.

⁴ Ztschr. f. physiol. Chem., 1907, 51, 71.

⁵ Jour. Immunology, 1917, 2, 573.

⁶ Amer. Jour. Syph., 1922, 6, 289.

proof box, for eighteen to twenty-four hours, turning the layers after the first ten to twelve hours. The cakes of dried material are now broken up, placed in an incubator over night and ground into a fine powder which is kept in tightly stoppered bottles of colored glass at room temperature. *Three or more hearts may be prepared at one time and a mixture of the powder used in preparing extracts. Rapid drying is essential to prevent decomposition which results in greatly increasing the hemolytic activity of the extracts.*

(b) Twenty-five grams of powdered muscle are extracted with about 100 c.c. of ether in a Soxhlet for eighteen hours; if this apparatus is not available the powder is extracted with 200 c.c. of ether in a tightly stoppered bottle at room temperature for five days, being shaken occasionally each day. The ether is carefully removed and saved for the time being in a tightly stoppered bottle.

(c) The powder is now dried by fanning for a few minutes or by spreading on a glass plate for several hours, placed in a bottle, and extracted with 200 c.c. of 95 per cent. alcohol in an incubator for four days.

(d) The alcohol is carefully decanted, poured into a flat shallow dish, and fanned dry. The residue is extracted with 30 to 50 c.c. of ether for the ether-soluble portion; this ethereal extract is covered and allowed to stand for an hour or two for the heavy insoluble particles to settle out.

The ethereal extract is now mixed with the ether of primary extraction and the mixture concentrated by fanning until reduced to about one-quarter volume or about 25 to 30 c.c. Six volumes or about 150 c.c. of pure acetone are now added to the concentrated ether, which throws down a whitish precipitate; the mixture is covered and placed aside for several hours or over night for the complete separation of the acetone-insoluble portions. On the following day the supernatant acetone is decanted and the sticky residue of acetone-insoluble lipoids removed and kept in acetone in a tightly stoppered wide-mouthed bottle for future use.

(e) The muscle powder is now extracted for a second time with 100 c.c. of absolute acetone-free ethyl alcohol in an incubator for six days, guarding against evaporation and shaking the mixture once or twice a day, and if possible for at least one day in a mechanical shaker; the extract is now filtered through fat-free paper.

(f) Pure cholesterol, 0.2 gram (Kahlbaum's preferred), and all of the acetone insoluble lipoids previously prepared are dissolved in 10 c.c. of pure ether and the cloudy brownish mixture slowly added to the filtered alcoholic extract and well shaken. The extract is now placed in the incubator for a few hours or over night, being shaken occasionally, and then placed in a refrigerator for a day or two; the light brownish precipitate is now removed by filtration through fat-free paper or by decanting the extract. The finished antigen is kept in a tightly stoppered brown glass bottle in a refrigerator; any precipitate forming after this time is left undisturbed.

7. Aqueous Extracts of *Pallidum* Culture.—This antigen was prepared by Noguchi, who used pure cultures of *Treponema pallidum* in ascites kidney agar. Preferably several strains should be used in the preparation, which corresponds quite closely to luetin. Cultures grown seven, fourteen, twenty-one, twenty-eight, thirty-five, and forty-two days are chosen and examined, and those that show the best growths in the agar columns are selected. The oil is poured off, the tubes cut just above the kidney, and the column of ascites agar between the piece of kidney and the oil removed with particular care, so as not to include the kidney or the oil. This substance is ground in a mill until the spirochetes show disintegration. The thick emulsion is then diluted with normal salt solution and heated to 60° C. for one-half hour; 0.4 per cent. phenol is added as a preservative, and the emulsion titrated for its anticomplementary dose. In conducting complement-fixation reactions with pallidum antigen one-half of the anticomplementary dose is used, and the serum must be inactivated.

Comparative Antigenic Values of Various Extracts.—For several years past I have been particularly interested in studying, from a practical standpoint, antigenic values of the extracts most commonly employed in the serum diagnosis of syphilis, and comparing them with suitable alcoholic extracts of syphilitic liver as a standard antigen.

For this purpose antigens were carefully chosen after titration, and only those were employed that were safely free from anticomplementary action and whose antigenic dose was known. A large number of serums

and cerebrospinal fluids from syphilitic and non-syphilitic persons were tested with numerous different extracts at the same time and under similar conditions. A suitable alcoholic extract of syphilitic liver was always included among the antigens in testing each serum or fluid, and the other extracts compared with it in determining their antigenic value.

In the following table the results of such studies, covering a period of two years, are given:

COMPARATIVE ANTIGENIC VALUES OF VARIOUS TISSUE EXTRACTS

EXTRACTS.	ANTIGENIC PROPERTIES AS COMPARED TO ALCOHOLIC EXTRACTS OF SYPHILITIC LIVER.				
	Equal.	Stronger.	Weaker.	Negative (positive with alcoholic extract of syphilitic liver).	Positive (negative with alcoholic extract of syphilitic liver).
Cholesterinized alcoholic extracts of human, pig, and beef heart.....	50.0	30.0	20.0
Acetone-insoluble lipoids.....	73.0	10.8	9.7	3.2	1.8
Alcoholic extract of pig and beef heart.....	71.1	1.9	13.4	5.7	7.6
Acetone extract of syphilitic liver.....	68.7	4.4	18.9	6.6	1.3
Alcoholic extract of normal liver	73.2	23.5	3.5	

The results of these studies have shown:

1. That cholesterinized alcoholic extracts of human, beef, and guinea-pig heart are far more sensitive than simple alcoholic extracts of syphilitic liver. Another peculiar feature of these antigens is the fact that in syphilis, if they react at all, they usually do so quite strongly.

2. That the addition of cholesterol to crude alcoholic extracts of syphilitic liver and of normal liver doubles their antigenic sensitiveness without materially increasing their anticomplementary and hemolytic action.

3. We have practically never found a serum that reacted negatively with a cholesterol extract and positively with an alcoholic extract of syphilitic liver. On the other hand, in about 20 per cent. of cases the cholesterinized antigens will react positively, whereas with the plain antigen of syphilitic liver the reactions are negative. In the majority of such instances the person was known to be luetic, but had received treatment and was regarded clinically as cured, or the serum was that of a long-standing and unrecognized case of lues.

4. It is highly important that cholesterolized extracts be carefully standardized, and that any serum, even if but slightly anticomplementary, be discarded.

5. In our experience repeated negative reactions with satisfactory cholesterinized antigens constitute the best evidences of the absence of lues or testify to the recovery from a luetic infection. The treatment of syphilis should be continued until the patient's serum reacts negatively with alcoholic extract of syphilitic liver, and finally with cholesterinized extracts. The disease cannot be regarded as cured until the reaction has remained negative for a year or two at least, and treatment must not be discontinued until this result is secured, or it is shown that the serum is "Wassermann fast" and that it is impossible to secure a negative reaction.

6. For the less experienced worker, or when but one antigen is being used in conducting the reaction, a properly prepared alcoholic extract of syphilitic liver is to be recommended. One drawback to the use of this extract is the difficulty of obtaining suitable tissues for the preparation of the antigen. It has been my practice for many years to preserve the livers of as many stillborn fetuses as I could obtain in 70 per cent. alcohol, and to discard them later unless on section they showed the presence of numerous spirochetes. These antigens are usually more sensitive than similar extracts of normal liver, but it is important to remember that not every extract is satisfactory simply because it is prepared of syphilitic tissues.

7. A suitable preparation of acetone-insoluble lipoids, prepared after the method of Noguchi, constitutes a sensitive, reliable, and satisfactory antigen. When properly titrated and standardized and used with inactivated serums this antigen may prove quite sensitive and safe. Noguchi, in his efforts to simplify the technic of the syphilis reaction, impregnated filter-paper with this antigen and allowed it to dry. This preparation is unstable and generally unsatisfactory. The antigen is best preserved in a stock bottle or in ampules, and is diluted with salt solution just before being used in the test. Under these conditions we have found these extracts to be quite stable.

8. Plain alcoholic extract of human, guinea-pig, and beef heart are easily prepared, are quite inexpensive, and when properly titrated serve as satisfactory antigens. Boas uses alcoholic extracts of human heart (Michaëlis) exclusively, and has found that they yield better results than alcoholic extracts of syphilitic liver. Garbat and others use and recommend similar extracts of guinea-pig heart.

9. Aqueous extracts of pure cultures of pallida have not thus far yielded results equal or superior to ordinary non-specific antigens. As compared with lipoidal extracts, they have generally yielded reactions that are much weaker, and in primary and secondary syphilis may react entirely negatively. Much is yet to be learned, however, of bacterial antigens in general, and the subject must be regarded as still in the experimental stage.

It may be said to be well proved that extract antigens in the syphilis reaction are not biologically specific, and need not be extracts of syphilitic tissues. An antigen cannot give reliable or satisfactory results unless it is carefully titrated and its properties determined. Antigens may serve as a frequent source of error when the complement-fixation reactions are conducted by those possessing insufficient knowledge of their good and bad properties. The test for the syphilitic reaction should not be undertaken by any one not competent to titrate and judge of the qualities of the antigen to be employed.

Method of Diluting Antigens.—As a general rule, all organic extracts must be diluted with normal salt solution before being used.

If extracts and diluent are mixed quickly, the emulsion is clear of slightly opalescent. If the diluent is added slowly to the organic extract, the resulting mixture becomes quite turbid and milky. As shown by Sachs and Roudoni,¹ the antigenic power of the extract is more marked with the turbid than when the clear or opalescent emulsion is used. *For this reason, in testing for the syphilis reaction the emulsion of organic extract should be made so as to secure the maximum amount of turbidity. The required amount of antigen is placed in a test-tube, and the salt solution is added slowly with a pipet; or the salt solution may be placed in a tube and the extract added drop by drop and gently shaking with each addition. This is the preferred method.*

Although with each new extract it may be necessary to titrate with

¹ Berl. klin. Wchn., 1908, 45, 1968.

various dilutions before one that is satisfactory is reached, experience has shown in the majority of instances that the following dilutions are usually correct:

1. Alcoholic extract of syphilitic liver: 1 part with 9 parts of salt solution. Extracts of German manufacture are usually diluted six or seven times.

2. Cholesterinized extracts and acetone-insoluble lipoids in methyl alcohol require higher dilution, as 1 part of extract with 19 parts of salt solution.

3. Plain alcoholic extracts of human, beef, and guinea-pig heart: 1 part and salt solution, 19 parts.

4. Alcoholic extract of human heart, prepared after the method of Boas (quick method), is used in lower dilution, as 1 part of extract with 9 parts of salt solution.

5. The alcoholic extract of heart reinforced with lecithin and cholesterin used by the author is diluted with varying amounts of salt solution as described in the text.

6. Aqueous extracts of syphilitic liver and extracts of pallida culture are used undiluted, or may require dilution with 4 parts of salt solution.

Although these emulsions will keep for a few days if placed in the refrigerator, it is advisable to make up only the amount required for immediate use, as freshly prepared emulsions are better than older ones.

Principles for the Titration of Antigens.—Three values are to be determined:

1. The anticomplementary unit, or that amount of antigen that in itself is capable of fixing or inactivating the complement.

2. The hemolytic unit, or that amount of antigen that in itself is capable of lysing red blood-cells. This action is probably due to the presence of certain lipoids and alcohol.

3. The antigenic unit, or that amount of antigen that serves to absorb or fix a certain and constant dose of complement with a definite amount of syphilitic serum.

1. *Anticomplementary Titration.*—(a) Emulsions should be prepared by adding the extract *slowly* drop by drop from a 1 c.c. pipet to the measured amount of saline and shaking gently. (b) Fresh non-syphilitic serum should be used, but is not absolutely necessary; a mixture of sera is preferable in order to equalize the content of natural hemolysins. The serum should be heated in exactly the same manner as in the main tests and used in an average amount. (c) The mixtures of antigen, serum, and complement should be given exactly the same primary incubation as used in the main complement-fixation tests; also receive the same amounts of hemolysin and corpuscles and secondary incubation. (d) The titration should be conducted with increasing amounts of a given emulsion with constant amounts of serum and complement *to ascertain the smallest amount of extract just producing beginning inhibition of hemolysis which is designated as the anticomplementary unit.*

2. *Hemolytic Titration.*—(a) An average amount of heated human- or guinea-pig-serum should be used. (b) Increasing amounts of antigen emulsion with a constant amount of serum and corpuscles should be incubated in exactly the same manner as the secondary incubation of the main complement-fixation tests *to ascertain the smallest amount of extract just producing beginning hemolysis which is designated as the unit.*

3. *Antigenic Titration.*—(a) Emulsions should be prepared by adding the extract *slowly* drop by drop from a 1 c.c. pipet to the measured amount

of saline and shaking gently. (b) A mixture of strongly positive syphilitic sera should be used; this serum should be heated in exactly the same manner as in the main tests and used in an average amount. (c) The mixtures of antigen, serum, and complement should be given exactly the same primary incubation as used in the main complement-fixation tests; also receive the same amounts of hemolysin and corpuscles and secondary incubation. (d) The titration should be conducted with increasing amounts of antigen with constant amounts of serum and complement *to ascertain the smallest amount of extract giving complete inhibition of hemolysis which is designated as the unit.*

In these titrations the *hemolytic system* should be exactly as employed in the main complement-fixation tests. The complement should be prepared in the same manner and used in the same number of units; likewise the hemolysin and corpuscles and the total volume should be the same.

VARIOUS METHODS FOR CONDUCTING THE SYPHILIS REACTION

First Method; the Original Wassermann Reaction.—The simplest technic, and the one best adapted for inexperienced workers, is the original Wassermann reaction, performed with alcoholic instead of aqueous extracts of syphilitic liver as antigen. It is true that this method is not an exact quantitative reaction, and that it is probably less delicate than some of the modified methods, but its advantages are that it is easy of manipulation, is readily learned, and is especially recommended for persons who perform these tests at irregular intervals, as false positive reactions are less likely to occur than when the more delicate methods are used.

Second Method; the Author's Modification of the Wassermann Reaction with Multiple Antigens.—In this method the technic is essentially the same as in the first method, except that three different antigens are used instead of one, namely, cholesterinized extract of normal heart, plain alcoholic extract of syphilitic liver, human or beef heart, and acetone-insoluble lipoids.

With strongly reacting serums all antigens may yield positive reactions. With the serums of long-standing or treated cases of syphilis the cholesterinized extracts may react strongly positive, whereas with the plain alcoholic extracts the reactions are weakly positive, or negative with one and positive with the other. In cases of syphilis that have received considerable treatment the reaction may be negative at first with the plain alcoholic extracts, and as treatment is continued it may finally be negative with the cholesterinized extracts. In a certain percentage of cases, especially those of old infections of the central nervous system, the reaction is positive with the cholesterinized extract and negative with the other extracts; strong reactions of this character usually indicate syphilitic infection.

Third Method; the Author's Modification of the Wassermann Test with Varying Amounts of Serum and Based Upon Studies in the Standardization of Technic.—This complement-fixation technic is based upon the results of nearly six years of continuous investigation upon the subject of standardization of technic.

The method of study pursued was to become acquainted with all existing methods by thoroughly reviewing the available literature and by means of personal communications and interviews with a large group of serologists and *submitting the whole to careful unbiased experiment, and choosing that proving best on the basis of actual trial.*

Requirements of a Standard Wassermann Reaction and How These Have Been Fulfilled by the New Technic.—In my opinion the essential requirements of a standard technic may be summarized¹ as follows: (1) As high degree of sensitiveness as is permissible with specificity; (2) practical specificity; (3) technical accuracy and uniformity in results; (4) yield a quantitative reaction; (5) simplicity in technic; (6) economy.

An attempt has been made to fulfil all of these requirements in the new technic by the following procedures:

Meeting the Requirement of Sensitiveness.—1. *By the use of a highly sensitive antigen.* This is of paramount importance and the new extract previously described² has been found almost free of hemolytic activity, very low in anticomplementary activity and very highly antigenic, permitting:

2. *By the use of large amounts of antigen.* At least ten antigenic units of the new antigen may be used with entire safety, this amount being six times or more less than the anticomplementary and hemolytic units and yielding very sensitive but true reactions. A large amount of time has been devoted to a study of different kinds of antigen, their manufacture,³ and preservation⁴; also to the proper amount to use from the standpoint of securing the greatest degree of sensitiveness consistent with true reactions.^{5, 6} With the new extract 5 to 10 antigenic units have proved to be the optimum amounts to use.

3. *By using relatively large amounts of serum and spinal fluid in the test.* This phase has required a great deal of investigation⁷ because there are limits to be placed upon the amounts to employ because: (1) The introduction of serum constituents other than antibody may interfere with complement fixation; (2) excessive amounts may result in non-specific reactions due to the anticomplementary activities of serum and spinal fluid, and (3) reduce sensitiveness by the introduction of natural hemolysin and hemagglutinins. To these may be added: (4) The question of economy.

Most serologists use 0.1 to 0.2 c.c. of serum, and 0.4 to 1.0 c.c. spinal fluid; the original Wassermann test requires 0.2 c.c. serum, and 0.4 c.c. spinal fluid. Calculated in relation to the amount of complement employed, the largest amount of spinal fluid used in the new test, namely, 0.5 c.c., corresponds to 1.5 to 2.0 c.c. in the original Wassermann test. Extensive trials have shown that for routine work the use of these amounts results in the greatest possible degree of sensitiveness to be expected on the basis alone of amounts of serum and spinal fluid employed.

4. *By heating sera for only fifteen minutes at 55° C. instead of for thirty minutes.* As stated in a previous paper⁸ we found it necessary in routine work to heat sera for the purpose of (1) removing anticomplementary substances; (2) removing the possibility of non-specific proteotropic reactions, and (3) removing native complement and depending upon a mixture of guinea-pig complements. These objects are secured by heating for fifteen minutes at 55° C. unless a serum is older than four days or improperly preserved, when thirty minutes may be required for the removal of antilysins. *Heating for fifteen minutes results in much less destruction of syphilis antibody, and has been found uniformly satisfactory when tests are conducted every three or four days, the specimen of blood kept in a refrigerator, and the serum left on the clot until used. Spinal fluids are used unheated.*

¹ Amer. Jour. Syph., 1919, 3, 1.

² Ibid., 1922, 6, 74.

³ Ibid., 1922, 6, 289.

⁴ Ibid., 1922, 6, 319.

⁵ Ibid., 1922, 6, 481.

⁶ Ibid., 1922, 6, 651.

⁷ Ibid., 1922, 5, 439.

⁸ Ibid., 1920, 4, 641.

5. *By using a mixture of guinea-pig complements prepared in a manner tending to increase sensitiveness to fixation.* As is well known, guinea-pig complement varies in fixability by syphilis antibody and tissue extracts¹; we have not found it necessary to titrate individual sera for fixability, a mixture meeting the requirements. Human complement also varies in fixability and explains why a syphilitic serum may give a falsely negative reaction in tests employing the patient's own complement; I am convinced that this may occur² and it constitutes an important reason for conducting the tests with a mixture of the sera of healthy guinea-pigs.

6. *By mixing serum and antigen for a brief period before the addition of complement in setting up the test; this appears to slightly increase the sensitiveness of reactions.*³

7. *By using a primary incubation of fifteen to eighteen hours in a refrigerator at 6° to 8° C.* This is of great importance from the standpoint of increasing the delicacy of reactions.^{4, 5, 6, 7} *It must be emphasized, however, that refrigerator incubation increases non-specific complement fixation and that the hemolytic system must be adjusted accordingly; a hemolytic system adjusted for conducting the primary incubation in a water-bath or thermostat for one hour is not likely to prove satisfactory for the refrigerator method.*

Refrigerator incubation of two hours or less with one hour water-bath is better than one hour in water-bath alone, but inferior to fifteen to eighteen hours in a refrigerator. The adoption of the latter necessarily requires two days for the conduct of tests; this is to be regretted, but the fact remains that it results in better work and is, therefore, recommended.

8. *By close adjustment of the hemolytic system in order to avoid excessive amounts of complement and hemolysin.*^{8, 9} This is accomplished by titrating both hemolysin and complement before the main tests. Guinea-pig complement occasionally contains natural hemolysin¹⁰ and this is adjusted for in the new technic by daily titration of hemolysin, the extra work and time required being negligible factors. Complement is titrated in the presence of the antigen, permitting a close adjustment of the dose to employ.

Extensive experiments have shown that *under these conditions and with a primary incubation of fifteen to eighteen hours at 6° to 8° C. it is necessary to use 2 full units of complement and 2 units of hemolysin in order to obtain sharp, clear, and decisive reactions without danger of non-specific results.*¹¹

9. *By using an antisheep or antioox hemolytic system.* I have reached this decision reluctantly and only after a very large number of comparative tests. There is so much *theoretic* evidence in favor of the antihuman hemolytic system that *only extensive comparative tests have shown most conclusively that with the new technic an antisheep system yields the best and most sensitive reactions.* An antioox system ranks second.

In the new test the question of the influence of natural antisheep hemolysin in the sera and complement is rendered practically negligible. Furthermore, the antisheep system permits the use of such powerful hemolysins easily prepared by the immunization of rabbits, that the amount of complement and hemolytic sera required are greatly reduced. This is an important factor, for it appears that the use of relatively large amounts of guinea-pig complement and rabbit hemolytic serum demanded by an anti-

¹ Amer. Jour. Syph., 1919, 3, 407.

² Ibid., 1919, 3, 541.

³ Ibid., 1921, 5, 290.

⁴ Ibid., 1920, 4, 675.

⁵ Ibid., 1921, 5, 30.

⁶ Ibid., 1921, 5, 44.

⁷ Ibid., 1921, 5, 63.

⁸ Ibid., 1920, 4, 518.

⁹ Ibid., 1920, 4, 616.

¹⁰ Ibid., 1920, 4, 484.

¹¹ Ibid., 1920, 4, 518.

human system as compared with an antishoop, introduce enough other serum constituents to reduce the degree of complement fixation by syphilis antibody and antigen. Whether or not this is the true explanation, the fact remains that actual comparative tests under rigid conditions have shown the superiority of the antishoop and the antiox hemolytic systems.

10. *By reading the reactions within three hours after the conclusion of the secondary incubation.* This permits the partial and sufficient settling of non-hemolyzed corpuscles for the purpose of accurate readings without allowing an excessive amount of hemolysin sometimes represented by natural antishoop hemolysin in a human serum^{1, 2} to continue as is apt to occur when the tests are placed in a refrigerator over night before the readings are made.³

Meeting the Requirements of Practical Specificity.—The phrase "practical specificity" is used purposely because the Wassermann reaction cannot be rendered biologically or absolutely specific for syphilis alone; positive reactions undoubtedly occur in frambesia or yaws.

However, the new test must avoid non-specific reactions due to avoidable errors in technic:

1. By close adjustment of the hemolytic system to a primary incubation of fifteen to eighteen hours at 6° to 8° C. in order to supply sufficient complement and hemolysin for non-specific fixation and yet to detect the slightest degrees of specific fixation by syphilis and antigen.

2. By careful titration of antigen under conditions rendering the dose employed suitable for a primary incubation of fifteen to eighteen hours at 6° to 8° C.

3. By incubating controls in every test and especially serum, antigen, and hemolytic controls to detect anticomplementary activities of serum and antigen or defects in the hemolytic system; also corpuscle controls to check the tonicity of the saline solution and fragility of cells. Test with sera from healthy normal and from syphilitic individuals should be included as positive and negative controls.

Meeting the Requirements of Technical Accuracy and Uniformity in Results.—This has been fulfilled in the new technic by the following procedures:

1. By adopting the principle that pipeting relatively large amounts of fluid (0.2 to 1.0 c.c.) tends to greater accuracy than measuring smaller amounts (less than 0.2 c.c.). This appears justified in view of the known inaccuracy of ordinary pipets and other measures, as well as a wide difference in the skill and care of individual workers. In the new technic the smallest amount of patient's serum or spinal fluid to be measured is never less than 0.2 c.c.; this permits of the use of 1 c.c. pipets divided into 0.1 c.c.

2. By using a total volume of 3 c.c. with sufficient corpuscles and test-tubes of suitable size to yield clear, sharp, and easily read reactions.

3. By using a reading scale⁴ furnishing hemoglobin in solution and non-hemolyzed corpuscles for reading the finer differences in the degree of hemolysis or no hemolysis.

In regard to uniformity in results it must be emphasized that the anticomplementary activity of serum or spinal fluid is very important in relation to reactions. For this reason tests conducted with portions of the same specimen of blood in different cities cannot be expected to yield absolutely similar results, nor even in the same city, if serologists vary in their method of preserving blood until the tests are conducted.

¹ Amer. Jour. Syph., 1920, 4, 111.

³ Ibid., 1920, 4, 135.

² Ibid., 1920, 4, 135.

⁴ Ibid., 1922, 6, 64.

Two or more serologists working in the same or different laboratories testing portions of a sample of blood or spinal fluid from one person should agree at least upon the question of positive or negative reactions; in my experience most variation occurs with serums yielding weakly positive reactions. Slight discrepancies in the reports on the degree of complement fixation must be expected, inasmuch as the personal equation plays an important part in reading the degree of hemolysis, as it does in matching colors in other lines of work, for example, in hemoglobin estimations and color reactions in general. Slight discrepancies, however, do no harm as long as the primary question of whether a serum does or does not yield a positive or negative result is untouched and particularly with serums yielding the borderline weakly positive or doubtfully negative reactions.

The new test has been found to fulfil this primary requisite, and furthermore has yielded remarkably similar reactions in the hands of different serologists working with portions of the same serum or spinal fluids in two different laboratories in Philadelphia.

Meeting the Requirements of a Quantitative Reaction.—This is an important requirement in relation to the Wassermann test as a serologic guide on the treatment of syphilis. The ordinary test employing a single dose of serum or spinal fluid is only roughly quantitative and is better designated as qualitative as previously described.¹

A complement-fixation test may be made quantitative by any of three procedures as follows:

(a) Using varying amounts of serum or spinal fluid with constant amounts of complement and antigen.

(b) Using varying amounts of complement with constant amounts of serum or spinal fluid and complement.

The first two are much more satisfactory than the third; the first has been adopted because most economic and equally satisfactory as the others.

In the new technic patient's serum is used in the following amounts: 0.1, 0.05, 0.025, 0.005, and 0.0025 with 0.1 c.c. in the serum control. Spinal fluid is used in amounts of 0.5, 0.25, 0.125, 0.0625, and 0.03125 c.c. with 0.5 c.c. in the control. Extensive trials with varying amounts of serum in the different stages of syphilis and with spinal fluids from cases of neuro-syphilis, have shown that these amounts are satisfactory. One object was to adopt as the largest doses of serum or spinal fluid amounts yielding the most sensitive reactions and for the smallest doses, amounts yielding less than total inhibition of hemolysis in the great majority of cases of syphilis.

Between these extremes are three graded doses making five in all, the control being the sixth tube of the series. Of course, a finer quantitative test can be secured by introducing eight tubes carrying 0.1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015 c.c. serum with 0.1 c.c. in the control, but six tubes has proved satisfactory and materially reduces both time and materials required. The reading scale permits reading the results with each of the five different amounts of serum or spinal fluid according to the + + + +, + + +, + +, + and - scale. This technic is quantitative, therefore, in two directions, namely, by using five graded amounts of fluid to be tested with five possible readings on each.

Meeting the Requirements of Economy.—This refers to both time and materials. From the standpoint of time required the new test cannot qualify as being economical; from the standpoint of materials it easily qualifies.

The new technic is not a short-cut method; I am convinced that the

¹ Amer. Jour. Syph., 1922, 6, 64.

principles involved in complement fixation are too intricate, the reagents too subject to variation, and our knowledge of the mechanism of the reaction too meager to permit the evolution of a short cut and simple test fulfilling the requirements of a standard test. Doubtless the time and labor involved for conducting the new test will prevent its adoption by many serologists, but I have endeavored to adhere to the principle that accuracy should never be sacrificed for speed and labor saving. The new technic provides for a quantitative and a qualitative test; I use the former routinely because it requires but little more time.

In so far as materials are concerned the quantitative test requires but 0.3 c.c. serum and 1.5 c.c. of spinal fluid; the qualitative test requires but 0.2 c.c. serum and 1.0 c.c. of spinal fluid.

In the new quantitative test 1 c.c. of guinea-pig serum is usually sufficient for examining 6 to 7 sera or spinal fluids; in the qualitative test this amount suffices for at least 15 sera or spinal fluids including all controls. In the original Wassermann test, which is a qualitative test only, 1 c.c. of complement is sufficient for testing 8 sera or spinal fluids including the usual controls. Complement is the most expensive reagent and the new test easily meets the requirements of economy in this and all other materials. The amounts of blood corpuscles and hemolysin required are so small as to not be worthy of discussion.

Meeting the Requirements of Simplicity.—As previously stated, simplicity is but a relative term in as much as the simplest technic is a complicated problem for the inexperienced and insufficiently trained worker, whereas, a more complicated technic is perfectly simple to the experienced serologist.

A new technic introduces only well-known principles and I hope will be accepted as relatively simple; certainly the test can be carried out by a careful and conscientious worker in any laboratory supplied with accurate glassware, a water-bath, and a refrigerator.

The simple examination of urine for albumin is a procedure capable of yielding different results in the hands of different workers; the Wassermann test requires a worker who has a working understanding of the principles, who refuses to compromise with something almost as good or almost satisfactory, and who conducts his or her work with a reasonable degree of accuracy and skill, refusing to sacrifice these for mere speed.

Fourth Method; the Author's Modification of the Wassermann Reaction Employing Varying Amounts of Complement.—It has previously been pointed out that the syphilis reaction is dependent upon the fact that while hemolytic complement may be rendered inactive or fixed by serum alone and organic extract alone, it is characteristic of syphilis that a mixture of serum and extract will absorb or fix more complement than the sum of the amounts absorbed by these two substances alone. In the foregoing methods no attempt has been made to measure the amount of complement absorbed by serum and antigen alone, but sufficient complement has been furnished to allow for this non-specific fixation, and we are content to show that the serum and antigen alone do not absorb enough complement to interfere with hemolysis, so that any inhibition of hemolysis may be interpreted as specific complement fixation.

Browning and Mackenzie and Thomsen have devised a technic whereby it is possible to estimate the actual amounts of complement absorbed, first, by the serum and antigen alone, and second by these two substances combined. The complement absorbed is measured in terms of hemolytic doses. This method consumes a little more time and more of the various reagents is required. It is, nevertheless, a good quantitative method, shows



FIG. 130.—TITRATION OF HEMOLYTIC COMPLEMENT.

The tube containing 0.4 c.c. of complement is the smallest amount producing complete hemolysis, and this amount is the *unit*.

exactly the degree of complement fixation in each case, and is especially valuable for research work rather than for routine purposes.

In conducting any complement-fixation test the following are essential factors if success is to be achieved: (1) Reliable reagents, particularly a good antigen must be had, for no matter how much care is exercised, good results cannot be secured with indifferent reagents; (2) the observer must possess a thorough working understanding of the underlying principles and particularly of the quantitative relations of the various reagents; (3) there must be an accurate adjustment of the hemolytic system; (4) he must have a careful, painstaking and accurate habit of pipeting small amounts. Accuracy should never be sacrificed for speed, as the latter is properly acquired only with experience.

TECHNIC OF THE FIRST METHOD

THE ORIGINAL WASSERMANN REACTION

This is the original Wassermann reaction, except that an alcoholic, instead of an aqueous extract of syphilitic liver, is used as antigen. This is the simplest of all technics, and, when properly performed, constitutes, in the final analysis, a reliable test and one especially adapted for those not constantly engaged in this work.

1. **Complement.**—Fresh clear serum (not over twenty-four hours old) of a healthy guinea-pig. Dilute 1 : 10 by adding 9 c.c. of sterile normal saline solution to each 1 c.c. of serum. Dose, 1 c.c. (= 0.1 c.c. of undiluted serum).

2. **Corpuscles.**—Sheep's blood washed three times and diluted to make a 5 per cent. suspension. For example, 1 c.c. of corpuscles in 19 c.c. of salt solution makes up sufficient for a number of tests.

3. **Hemolytic Amboceptor.**—Serum of a rabbit immunized with washed sheep's corpuscles. As stated elsewhere, this serum is heated to 55° C. for half an hour, and an equal part of chemically pure glycerin is added. Mix well and preserve in sterile 1 c.c. ampules. Each ampule will, therefore, contain 0.5 c.c. of serum. One stock dilution is prepared in such manner that about 0.2 c.c. represents one hemolytic unit. One may otherwise prepare a whole series of flasks with various dilutions, and in making a titration to use 1 c.c. of each dilution; I have found it much more accurate, simple, and economical, however, to prepare one stock dilution, *which is titrated with each complement and corpuscle suspension before each day's work.* For example, if a serum is known to have a titer of 1 : 2000, an ampule (0.5 c.c. of serum) is diluted with 200 c.c. of salt solution; this gives a dilution of serum approximately 1 : 400, of which 0.2 represents one hemolytic unit. The titration must be repeated each time to make sure of this, because the complement of different pigs may vary in activity, and the chief object is to adjust the hemolysin and complement to each other.

Titration of Hemolysin.—Into a series of six test-tubes place increasing amounts of the amboceptor dilution: 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 c.c. Add 1 c.c. of complement (1 : 10) and 1 c.c. of corpuscle suspension to each tube, and sufficient salt solution to make the total volume in each tube about 4 c.c. Shake gently and incubate for one hour at 37° C. At the end of this time the tube showing just complete hemolysis contains one hemolytic dose, or unit of amboceptor. In the tests double this amount, or two units, is used.

The amboceptor titration is very important. Under no circumstances should the same dose be used day after day without titration, because the complement of different guinea-pigs may vary in its activity, and these

variations would be detected and would be adjusted in this titration. For example, with a weaker complement the dose of amboceptor required to effect complete hemolysis becomes higher; each new corpuscle suspension may also vary slightly in the actual number of cells contained in 1 c.c., but this makes no difference when each suspension is titrated with the complement and amboceptor to be used in the day's work. This titration is set up first, and while it is in the incubator, the main tests are arranged.

4. Antigen.—Alcoholic extract of syphilitic liver or acetone-insoluble lipoids of proved value may be used. It is well to estimate just how much antigen will be required for the tests on hand, so that no waste will occur, as fresh emulsions are better than old ones carried over from day to day. The dose should be at least double the titrated antigenic unit, or one-fourth of the anticomplementary dose. For instance, if an alcoholic extract of syphilitic liver diluted 1 : 10 is found on titration to be perfectly antigenic in doses of 0.2 c.c., and not anticomplementary in amounts under 2 c.c., then 0.4 c.c. may be used in making the tests, as this amount is still about five times less than the anticomplementary dose, and well within the range of safety against non-specific complement fixation. If 10 tests are to be made, then at least 4.4 c.c. of diluted antigen are required, including sufficient for the antigen control, or in round numbers, 0.5 c.c. of antigen plus 4.5 c.c. of salt solution *slowly added in order to secure the maximum turbidity*.

5. Serum.—This should be fresh and clear and heated in a water-bath to 55° C. for half an hour before using. The temperature should not go above 56° C. nor below 55° C. Dose, 0.2 c.c.

6. Cerebrospinal Fluid.—This should be fresh and free from blood. It is used unheated, as spinal fluid contains little or no hemolytic complement. The dose should be at least four times that of the serum, or 0.8 c.c.

The Test.—A front and a rear tube for each serum are placed in a rack. Each tube is marked plainly with the patient's name or initials, and in addition the front tube is marked with the number of the antigen or with the letter "A," or the word "antigen" is written on it, the rear tube being marked "control" (serum control). The necessity for carefully marking each tube is nowhere more important than in conducting Wassermann reactions with a number of serums, as the slightest error or lapse of memory may result in confusion and prove to be quite a serious matter.

In each series of reactions the serum from a known case of syphilis that has given a positive reaction and the serum of a known non-syphilitic person are included as positive and negative controls respectively.

Into each front tube the proper dose of antigen is placed; to the front and rear tubes 0.2 c.c. of the patient's serum is added. To all tubes 1 c.c. of the complement (1 : 10) and sufficient normal salt solution are then added to bring the total volume in each to about 3 c.c.

The rear tube of each set is the serum control; the positive and negative serums are treated in just the same manner as the patient's serum. In addition to these there are three other important *controls* that should not be omitted:

1. *The antigen control:* Dose of antigen plus 1 c.c. of complement (1 : 10) and a sufficient quantity of salt solution.

2. *Hemolytic system control:* 1 c.c. of complement (1 : 10) and 2 c.c. of salt solution.

3. *Corpuscle control:* 1 c.c. of corpuscle suspension plus 3 c.c. of salt solution.

Each tube is gently shaken and incubated at 37° C. for an hour, when two units of amboceptor and 1 c.c. of corpuscle suspension (5 per cent.)

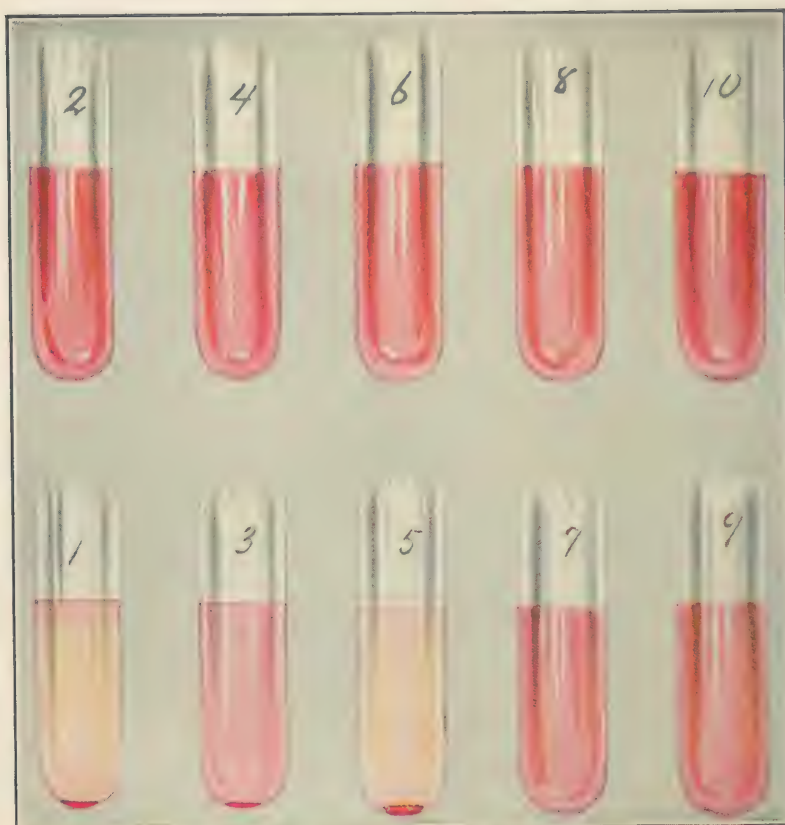


FIG. 131.—WASSERMANN REACTION (FIRST METHOD).

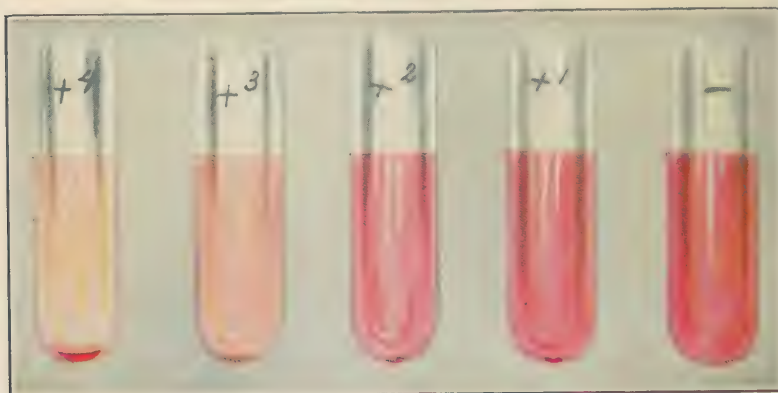


FIG. 132.—READING THE WASSERMANN REACTION.

are added to each tube except the corpuscle control. Tubes are shaken and reincubated for an hour or an hour and a half, depending upon the hemolysis of the serum controls, after which a preliminary reading is made and recorded. With partially positive reactions the tubes may be centrifuged in order to read the relative amounts of hemolysis, and the final reading made at once, or the tubes may be placed in the refrigerator (just above freezing-point) and the final readings made the next morning.

SCHEME FOR CONDUCTING A WASSERMANN REACTION (FIRST METHOD). (SEE FIG. 131.)

UNKNOWN SERUM, MR. B.	UNKNOWN CEREBROSPINAL FLUID, MR. C.	KNOWN POSITIVE SYPHILITIC SERUM.	KNOWN NEGATIVE NORMAL SERUM.	CONTROLS.
2. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	4. Cerebrospinal fluid, 0.8 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	6. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	8. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:20) +. Salt solution (q. s. 3 c.c.).	10. Antigen control: Antigen, 0.4 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).
1. Antigen, 0.4 c.c. +. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	3. Antigen, 0.4 c.c. +. Cerebrospinal fluid, 0.8 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	5. Antigen, 0.4 c.c. +. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).	7. Antigen, 0.4 c.c. +. Serum, 0.2 c.c. +. Complement (1 c.c. of 1:20) +. Salt solution (q. s. 3 c.c.).	9. Hemolytic control. Complement (1 c.c. of 1:10) +. Salt solution (q. s. 3 c.c.).

Tubes are shaken gently and incubated at 37° C. for an hour, after which two hemolytic doses of amboceptor and 1 c.c. of corpuscle suspension are added to each. They are then gently shaken and reincubated for an hour or an hour and a half, after which a preliminary reading is made.

All the tubes in the rear row (upper row in table) (serum controls), the antigen and hemolytic system controls, and the front tube with the negative normal serum, are completely hemolyzed. The front tube with the unknown serum and cerebrospinal fluid and the positive serum control show inhibition of hemolysis or positive reactions.

This scheme illustrates the technic employed with an unknown serum and cerebrospinal fluid. The proper dose of diluted antigen is taken as 0.4 c.c., and two doses of hemolytic amboceptor determined by titration as equivalent to 0.4 c.c. of the stock dilution.

Reading and Recording the Wassermann Reaction.—1. The hemolytic system control is inspected first. It should show complete hemolysis, indicating that the complement and amboceptor were active and have been used in sufficient amounts. If a few corpuscles are found in the bottom of the tube, some error in pipeting has probably occurred, too many corpuscles or too little complement or amboceptor having been introduced.

2. The corpuscle control should show no hemolysis, indicating that the solution is isotonic and that the corpuscles are not unduly fragile.

3. The antigen control should show complete hemolysis, indicating that the dose used was not anticomplementary. If this tube shows incomplete hemolysis, due to the anticomplementary action of the antigen, all the front two tubes will also show some inhibition of hemolysis, due to this non-specific complement fixation, and it is necessary to repeat the tests with another extract.

4. The rear tubes of all serums should be completely hemolyzed, indicating that the serums were practically free from anticomplementary action as previously stated, most antigens and serums are usually very slightly anticomplementary if small amounts of complement are used with a close single unit of amboceptor, but in this technic the complement and 2 units of amboceptor are sufficient, under ordinary circumstances, to offset this influence. If, however, a serum is more than normally anticomplementary, the rear tube will show some inhibition of hemolysis, and, of course, in the front tube a similar inhibition, and probably to a greater degree, will be seen. If the serum is very slightly anticomplementary and the front tube shows complete inhibition of hemolysis, the reaction is in all probability positive. If the rear tube, however, shows marked inhibition of hemolysis, indicating that it is highly anticomplementary, the result cannot be determined, but a retest with fresh serum must be made. *This indicates the great importance of the "serum control," and it may be stated that a test should never be made without it.*

5. The front tube containing the known syphilitic serum should show inhibition of hemolysis, indicating that the extract possesses antigenic properties.

As the complement is "fixed" by the syphilis antibody and extract, hemolysis could not occur when the corpuscles and amboceptor were added. If a portion of the complement is fixed by antibody and extract, then the unfixed portion will hemolyze some of the corpuscles, the reaction being moderately positive, slightly positive, etc., depending upon the degree of hemolysis that takes place. This illustrates the importance of observing exactness in pipeting, and the great influence of quantitative factors in testing for the Wassermann reaction, for if an excess of complement is used, there may be sufficient for all the syphilis antibody, and enough unbound complement to hemolyze all the corpuscles. In this manner a false negative reaction will result. Corpuscles and sufficient hemolytic amboceptor are added merely in order to test for any free complement. Under proper conditions a total lack of hemolysis indicates that there is no free complement, but that it has been fixed by syphilis antibody and extract, constituting a positive reaction (+ + + +). Complete hemolysis indicates that complement was not bound and that syphilis antibody was, therefore, absent from the fluid tested—a negative reaction (—). Partial hemolysis indicates that a portion of the complement has been fixed by smaller amounts of syphilis antibody and of the extract, yielding partially positive reactions (+ + +; + +; +; ±).

6. The front tube containing the known normal serum should show complete hemolysis because, in the absence of syphilis antibody, the complement remains free to hemolyze the corpuscles with the hemolytic amboceptor.

7. Various methods have been proposed for recording the results of hemolytic tests. The following scheme, after Citron, is widely used (Fig. 132).

- ++++ = complete inhibition of hemolysis = strongly positive.
- +++ = 75 per cent. inhibition of hemolysis = moderately positive.
- ++ = 50 per cent. inhibition of hemolysis = weakly positive.
- ± = 25 per cent. inhibition of hemolysis = very weakly positive.
- ± = less than 25 per cent. inhibition of hemolysis = delayed hemolysis or doubtful reaction.
- = complete hemolysis = negative reaction.

Under the third method a scale is given that is easily prepared for making these readings. However, after some experience they are readily made, and at first should be attempted only after the non-hemolyzed corpuscles have been centrifuged or allowed to settle to the bottom of the tube. As stated elsewhere, this method is not an accurate measure of the amount of syphilis antibody, but constitutes a relative and convenient gage of value within certain limits. In reporting reactions to the clinician, the plus signs should not be used, or if used, should be interpreted by the terms "strongly positive," "weakly positive," etc.

TECHNIC OF THE SECOND METHOD

AUTHOR'S MODIFICATION OF THE WASSERMANN REACTION WITH MULTIPLE ANTIGENS

Practically the same technic is used in this as in the first method, except that three different antigens, instead of one, are used with each serum, for the reasons previously stated; for economy the amounts of each reagent are just one-half those originally employed.

This method can be strongly recommended, as it is simple, accurate, and reliable. Although a little more work is demanded and a larger quantity of the various reagents is required, the results warrant the expenditure of a little more labor, and the second objection is readily overcome by using half the quantities prescribed in the original Wassermann technic, as given in the first method.

1. **Complement.**—Fresh clear sera of two or more guinea-pigs collected as previously described. If only a small number of tests are to be done sufficient blood may be obtained by bleeding two or more pigs from the heart as described on p. 37. Dilute the serum 1 : 20 (1 c.c. serum + 19 c.c. saline solution).

2. **Corpuscles.**—Sheep's corpuscles washed three times and made up into a 2.5 per cent. suspension with saline solution as previously described.

Hemolysin.—Antisheep hemolysin *titrated each time* the tests are conducted.

The serum is so diluted that the unit will be approximately 0.1 to 0.2 c.c. In a series of 6 test-tubes place increasing amounts of this stock solution as follows: 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 c.c. Add 1 c.c. complement 1 : 20 and 1 c.c. of 2.5 per cent. corpuscle suspension. Mix gently and place in a water-bath at 38° C. for one hour. The unit is the smallest amount showing complete hemolysis and 2 units are employed for the antigen titrations and main tests.

In conducting routine tests by this method the hemolysin titration may be put up last and incubated along with the primary incubation of the main tests; at the end of the hour the unit is read and 2 units added to the tubes of the main test.

3. **Antigens.**—I generally use the following *three antigens*: (1) A cholesterinized alcoholic extract of human heart; (2) alcoholic extract of syphilitic liver or alcoholic extract of heart; (3) acetone-insoluble lipoids.

As previously stated, these extracts are used in amounts equal to from two to four times their titrated antigenic unit, providing these doses are at least four times smaller than the anticomplementary units. The amount of each antigen required for the work at hand is calculated, placed in test-tubes, and slowly diluted with the requisite amount of salt solution to secure maximum turbidity of the emulsions.

1. *Anticomplementary Titration.*—*The antigen extract is diluted 1 : 10 by placing 1 c.c. in a test-tube and slowly adding 9 c.c. of normal salt solution or 1 : 20 (1 c.c. antigen + 19 c.c. saline).*

Increasing amounts of this emulsion are placed in a series of test-tubes: 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.5, and 2 c.c. To each tube are now added 1 c.c. of the diluted complement serum (= 0.05 c.c. undiluted serum), and sufficient normal salt solution to bring the total volume up to 3 c.c. Shake each tube gently and incubate for one hour at 37° C. Then add to each tube 1 c.c. of the corpuscle suspension and a dose of amboceptor equal to 2 units, as just determined by previous titration. Shake gently and reincubate for another hour and a half, when a preliminary reading of the results may be made. *That amount of antigen that shows beginning inhibition of hemolysis is regarded as the anticomplementary unit.* The final readings are made after the tubes have stood over-night in a refrigerator at low temperature (Fig. 133).

This titration may also be made in the presence of normal serum, although this is not absolutely necessary. *The serum must be perfectly fresh, and must be that from a person known to be free from lues.* It is inactivated by heating to 55° C. for half an hour, and 0.2 c.c. is added to each tube. Complement and salt solution are now added, and the titration conducted in the manner just described. Normal serum may absorb a small amount of complement in itself, and hence a titration conducted with serum may show a slightly lower anticomplementary dose.

The following controls are included:

1. A *hemolytic system control*, containing the complement, corpuscles, and amboceptor in the same amounts as were used in conducting the titration. This control should show complete hemolysis.

2. A *serum control*, which is the same as the hemolytic system control plus 0.2 c.c. of the serum. This should show complete hemolysis, and indicates that the serum was not anticomplementary. This control test should never be omitted.

3. A *corpuscle control*, including 1 c.c. of the corpuscles in salt solution. This tube should show no hemolysis.

The following table gives the results of a titration with an alcoholic extract of syphilitic liver diluted 1 : 10 (see Fig. 133).

ANTICOMPLEMENTARY TITRATION OF A TISSUE EXTRACT

TUBE.	ANTIGEN (1 : 10), C.C.	COMPLEMENT (1 : 20), C.C.	NORMAL SERUM, C.C.	Sufficient salt solution to bring the total volume to 3 c.c.; tubes shaken and incubated one hour.	ANTI-SHEEP HEMOLY- SIN UNITS.	SHEEP'S CORPUS- CLES 2.5 PER CENT., C.C.	RESULTS.
1....	0.2	1	0.2		2	1	Hemolysis.
2....	0.4	1	0.2		2	1	Hemolysis.
3....	0.6	1	0.2		2	1	Hemolysis.
4....	0.8	1	0.2		2	1	Hemolysis.
5....	1.0	1	0.2		2	1	Hemolysis.
6....	1.2	1	0.2		2	1	Slight inhibition of hemolysis.
7....	1.5	1	0.2		2	1	Marked inhibition of hemolysis.
8....	2.0	1	0.2		2	1	Complete inhibition of hemolysis.
9....	Control.	1	...		2	1	Hemolytic control: complete hemolysis.
10....	Control.	1	0.2		2	1	Serum control: complete hemolysis.

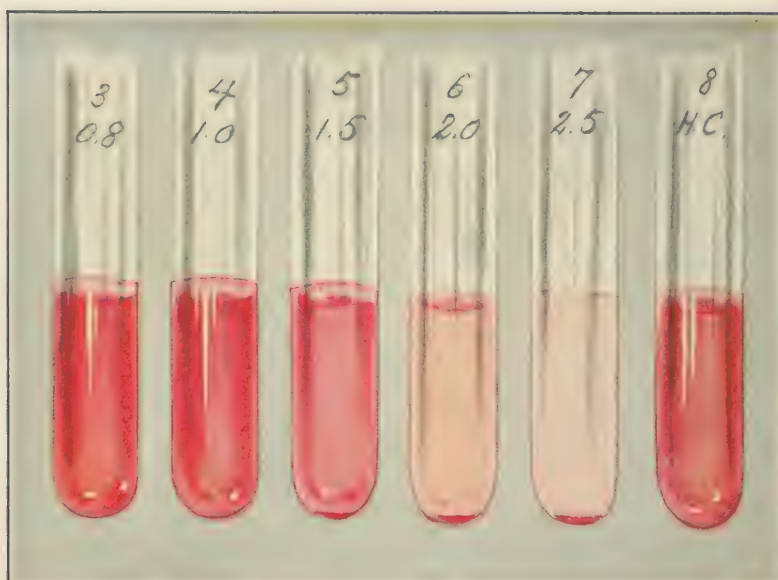


FIG. 133.—TITRATION OF ANTIGEN FOR ANTICOMPLEMENTARY UNIT.



FIG. 134.—TITRATION OF ANTIGEN FOR ANTIGENIC UNIT.

In Fig. 133 the tube containing 1.5 c.c. of antigen shows slight inhibition of hemolysis, and this amount is the *anticomplementary unit*.

In Fig. 134 the tube containing 0.15 c.c. of antigen is the smallest amount producing complete inhibition of hemolysis, and is the *antigenic unit*.

In this titration tube No. 6, containing 1.2 c.c. of the antigen emulsion showed beginning inhibition of hemolysis and was recorded as the anticomplementary dose.

2. *Hemolytic Titration*.—As previously mentioned, organic extracts are capable in themselves of hemolyzing red cells; this is due to the hemotoxic action of lipoids and alcohol. Extracts of organs that have undergone advanced autolysis and decomposition are very likely to be hemolytic.

Serum exerts an inhibiting influence on the lytic action of an organic extract. Hence the hemolytic dose of an extract depends largely on whether or not complement serum is used in the titration.

When an organic extract is titrated in the presence of complement, the hemolytic dose is higher than the anticomplementary dose. In the foregoing titration 3 c.c. of the extract emulsion showed beginning hemolysis, and when 4 c.c. was used, hemolysis was complete. These large amounts of emulsion give the tube contents quite a milky appearance, but close inspection shows that all the cells are broken up.

As a general rule, the hemolytic titration is not absolutely necessary. It may be conducted with the anticomplementary titration by adding another tube or two to the foregoing series, with higher doses of extract; or this titration may be conducted separately, and without complement hemolysin, by using the same doses of antigen with 1 c.c. of corpuscle suspension and sufficient salt solution to bring the total volume in each tube up to 3 or 4 c.c.

3. *Antigenic Titration*.—As previously stated, this titration is not absolutely necessary, as one-fourth the anticomplementary dose of an extract may be used in the main test. For instance, in the foregoing titration 0.3 or 0.4 c.c. may safely be used in making the test for the syphilitic reaction. Different extracts vary, however, in their antigenic value. Some may be highly anticomplementary and have a comparatively low antigenic value; purer extracts, such as acetone-insoluble lipoids or cholesterolized alcoholic extracts of heart, are largely free from anticomplementary action, and at the same time possess a high antigenic value. *It is advisable, therefore, to use an antigen whose full antigenic as well as anticomplementary doses are known, for, while it is necessary to use sufficient antigen, it is not advisable to use a larger amount than is necessary.*

For this titration all antigens except alcoholic extracts of syphilitic liver, should be diluted 1 : 20 with normal salt solution. Usually the antigenic unit is so much lower than the anticomplementary unit that it is best determined with a more dilute antigen.

The titration is conducted in a manner similar to the anticomplementary titration except that 0.2 c.c. of fresh and inactivated serum from a known and untreated syphilitic person is added to each tube. Increasing doses of antigen, patient's serum, and complement are mixed, shaken, and incubated for one hour. Two units of hemolysin and corpuscles are then added, the tubes shaken and incubated for another hour, after which the preliminary reading is made. The final reading is taken after the tubes have been placed over night in a refrigerator at low temperature. *That amount of antigen that shows just complete inhibition of hemolysis is taken as the antigenic unit (Fig. 134). In conducting the syphilis reaction two to four times this unit is used, providing that these amounts are at least four or five times less than the anticomplementary dose.* This larger antigenic dose is advisable, because the exact unit may not be sufficient with serums containing but small amounts of syphilis antibody such as those of treated or long-standing cases of lues.

The following table illustrates this titration with the same alcoholic extract of syphilitic liver (see Fig. 134):

ANTIGENIC TITRATION OF A TISSUE EXTRACT

TUBE.	ANTI-GEN 1 : 10, C.C.	SYPHI-LITIC SERUM (INACTIVE) C.C.	COM- PLE- MENT 1 : 20, C.C.	Sufficient salt solution to bring the total volume to 3 c.c.; tubes shaken and incubated one hour.	ANTI-SHEEP HEM- OLYSIN, DOSES.	SHEEP COR- PUSCLES 2.5 PER CENT., C.C.	Tubes shaken and incubated one hour, preliminary reading.	RESULTS.
1.....	0.05	0.2	1		2	1		Slight inhibition of hemolysis.
2.....	0.01	0.2	1		2	1		Marked inhibition of hemolysis.
3.....	0.15	0.2	1		2	1		Complete inhibition of hemolysis; <i>unit</i> .
4.....	0.2	0.2	1		2	1		No hemolysis.
5.....	0.25	0.2	1		2	1		No hemolysis.
6.....	0.3	0.2	1		2	1		No hemolysis.
7.....	Control.	0.2	1		2	1		Serum control: hemolysis.
8.....	Control.	0	1		2	1		Hemolytic control: hemolysis.

In this instance 0.15 c.c. of the emulsion represents the antigenic unit. In performing the Wassermann reaction 0.3 or 0.4 c.c. was used, and these amounts were about one-fourth the anticomplementary dose.

It is not unusual to find cholesterinized alcoholic extract and acetone-insoluble lipoids perfectly antigenic in 0.05 c.c. of a 1 : 20 dilution, and not anticomplementary under 1 or 2 c.c. of a 1 : 10 dilution. In these instances four times the antigenic dose, or 0.2 c.c. can be used, and yet this amount is at least ten times smaller than the anticomplementary dose—a condition of affairs that constitutes a safe and desirable antigen.

Each new antigen should be tested with a number of serums and controlled by an older antigen of known value before being finally accepted as satisfactory.

Antigen containers should be well stoppered and kept in the refrigerator. Deterioration may set in suddenly, and they should, therefore, be retitrated every few weeks.

4. **Serum.**—Heated in a water-bath to 55° C. for thirty minutes; dose 0.2 c.c. This amount is placed in each of the three tubes carrying the antigens and in the serum control.

5. **Cerebrospinal Fluid.**—Used unheated. Dose, 0.8 c.c. with each antigen; 1.0 c.c. may be used in the control.

The Test.—For each serum and spinal fluid four test-tubes are arranged in a row and marked with the patient's name or initials. The first tube is marked "C. H.," and receives the cholesterinized heart extract; the second tube is marked "S" for the alcoholic extract of syphilitic liver or plain alcoholic extract of heart; the third is marked "A" for acetone-insoluble lipoids, and the fourth is not marked at all or simply marked with the letters "S. C." (serum control).

To each of the four tubes 0.2 c.c. of the patient's serum is added, or 0.8 c.c. of cerebrospinal fluid.

To each tube 1 c.c. of the diluted complement (1 : 20) and sufficient salt solution to bring the total volume in each up to 3 c.c. are now added.

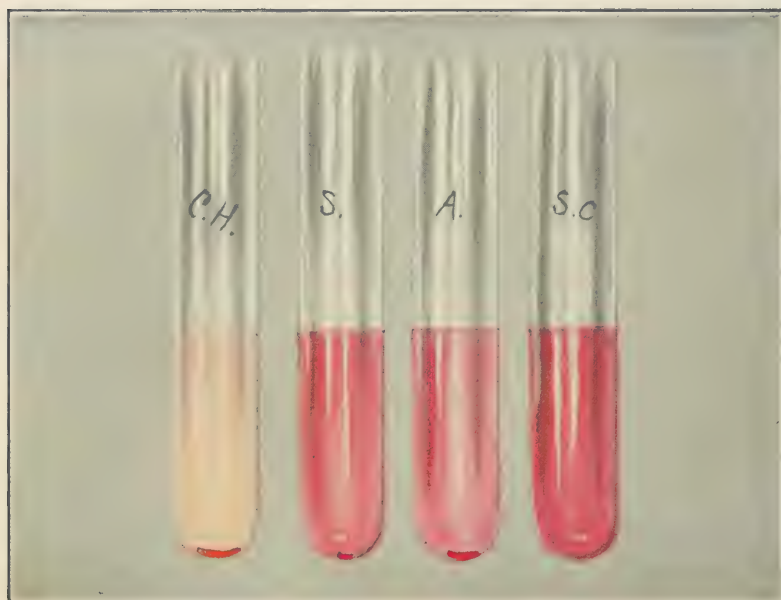


FIG. 135.—WASSERMANN REACTION (SECOND METHOD).

Shows a ++++ reaction with the cholesterinized extract (C. H.); a + reaction with the alcoholic extract of syphilitic liver (S), and a ++ reaction with the extract of acetone-insoluble lipoids (A).

Controls.—A known *positive* and *negative* serum should be included, unless one is performing a large number of tests with reliable antigens every week, in which case, among many serums, a few at least are likely to be positive. Under these circumstances these controls may be omitted; as a general rule, however, they should be included.

To the *hemolytic system control* tube 1 c.c. of complement dilution, and 2 c.c. of salt solution are now added. *Three antigen control tubes* are set up for each antigen with the dose employed, plus 1 c.c. of complement dilution and sufficient salt solution to make the total volume about 3 c.c. The *corpuscle control* receives 1 c.c. of the suspension plus 3 c.c. of salt solution.

All the tubes are shaken gently and placed in a water-bath for an hour at 37° C. Instead of the water-bath an incubator at 38° C. may be employed for one hour (not less) for the primary and secondary periods of incubation. At the end of primary incubation 2 units of the amboceptor and 1 c.c. of the corpuscles are added to each tube except that containing the corpuscle control. Each tube is shaken gently and reincubated for an hour or longer, depending upon the hemolysis of the controls, when the readings are made. By making the readings at this time the influence of an excess of hemolysin due to the presence of natural antisheep hemolysin in the human sera is avoided and lesser degrees of complement fixation detected, which may become completely hemolyzed if the tubes are set aside over night.

Reading the Results.—The readings are made in the same manner as described in the first method, the controls always being inspected first. The hemolytic, antigen, and serum controls and known negative serum tubes should all be hemolyzed. The antigen tubes containing the positive syphilitic serum should not be hemolyzed. Results with the unknown serums are dependent upon whether or not the serums are luetic, and if they are, upon the quantity of syphilis antibody present.

With strongly positive serums there is complete inhibition of hemolysis with all three antigens. With serums of long-standing or treated cases of syphilis containing smaller amounts of antibody the reaction with the cholesterinized extract is usually strongly positive, whereas with the other two antigens the degree of inhibition of hemolysis is less marked and variable (see Fig. 135). In from 15 to 20 per cent. of cases the cholesterinized extract shows a 50 per cent. or more inhibition of hemolysis, whereas with the other two antigens the reactions are negative. In our experience the majority of such serums were taken from patients giving a frank history of syphilis of many years' standing and from known cases undergoing treatment, further therapy being indicated until the reaction finally becomes negative when cholesterinized extracts are used. In a small proportion of cases a feebly positive reaction of 25 per cent. or less inhibition of hemolysis may be found with the cholesterinized extract alone. Many of these reactions occur with serums of treated cases of syphilis; on the other hand, a similar reaction may occur with about 5 per cent. of normal serums, so that if the history and clinical conditions are clearly negative, a slight degree of inhibition of hemolysis (5 to 10 per cent.) with the cholesterinized extract and marked hemolysis with the other two antigens may be interpreted as a negative reaction.

After a new antigen has been prepared and titrated, it should be tested out in this manner by placing it in the series along with at least two other older antigens of proved value, and used in the examination of a large number of serums before it is finally accepted as reliable.

TECHNIC OF THE THIRD METHOD

AUTHOR'S MODIFICATION OF WASSERMANN REACTION BASED UPON STUDIES IN THE STANDARDIZATION OF TECHNIC

Glassware and Apparatus.—Good *pipets* are essential; certified pipets are best, of course, but it is advisable to have a separate 1 c.c. pipet for each serum and the item of expense is apt to be prohibitive. As previously stated, the new technic calls for pipeting amounts from 0.2 to 1.0 c.c. in order to reduce error due to inaccurate pipets.

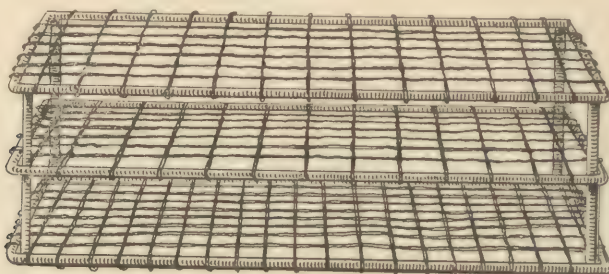


FIG. 136.—A WIRE RACK USED BY THE AUTHOR FOR COMPLEMENT-FIXATION TESTS; CARRIES 72 TUBES.

One c.c. pipets graduated to the tips are preferred as long as the tips are not chipped.

Five and 10 c.c. pipets divided into 0.5 cm. are also required (Fig. 4).

The *test-tubes* should have rounded bottoms, no lips, and measure 85 mm. in length with an internal diameter of 11 to 13 mm. It is important that the diameter be within these limits on account of the color scale. Their size is shown in Fig. 140.

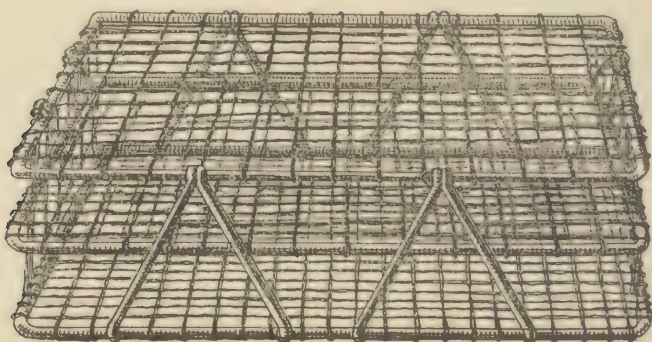


FIG. 137.—A LARGER WIRE RACK USED BY THE AUTHOR FOR COMPLEMENT-FIXATION TESTS; CARRIES 144 TUBES.

For mixing large volumes, as in the preparation of corpuscle suspension or dilutions of complement serum, *volumetric flasks* rather than the ordinary graduated cylinders should be used because of greater accuracy. The *glass-stoppered graduated cylinders* (50 to 100 c.c. capacity) are more convenient, however, for measuring intermediate amounts, and may be used if carefully selected on the basis of accuracy in graduations. For

measuring any amount of fluid under 50 c.c. it is better to use an accurate 10 c.c. pipet and reserve the graduated cylinders or flasks for measuring larger volumes.

It is imperative that all glassware including new glassware should be chemically clean, that is, free of all traces of acids or alkalies and preferably sterile; test-tubes do not require cotton plugs, but may be sterilized in baskets open ends down. A full description of technic for cleaning test-tubes and pipets is given on p. 7.

Test-tube Racks.—Each test requires six test-tubes. Galvanized wire racks (Fig. 136) carrying 12 rows of six tubes each have been found very serviceable; also racks carrying 24 rows of six tubes each (Fig. 137).

Water-bath.—A simple and inexpensive water-bath for heating sera and conducting the secondary incubation is shown in Fig. 138. A much simpler pan made by any tinsmith is shown in Fig. 139. When carrying water to the depth of 8 cm. the temperature of either pan may be maintained at 55° or 37° C. with very little care and attention.

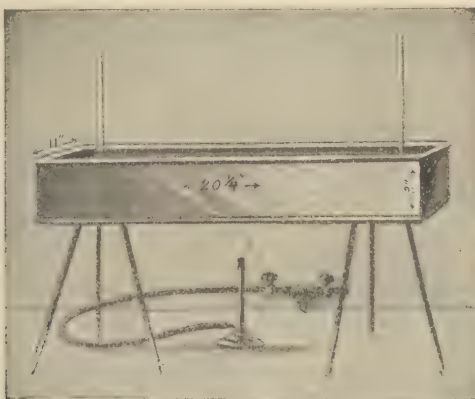


FIG. 138.—A VERY SIMPLE AND EFFICIENT WATER-BATH USED BY THE AUTHOR FOR THE INACTIVATION OF SERA AND FOR SECONDARY INCUBATION. Size is indicated. (Amer. Jour. Syphilis 1922, 6, 92.)

Refrigerator.—Any refrigerator maintaining a temperature of between 6° and 8° C. suffices for the primary incubation.

Saline Solution.—Sodium chlorid (0.85 per cent.) in water prepared as described on p. 9.

Corpuscles (Indicator Antigen).—*Two per cent. suspension of freshly collected and washed sheep corpuscles; dose 0.5 c.c.*

Abattoir blood may be used, but occasionally a worker will encounter corpuscles more resistant to hemolysis than average cells; for this reason it is better to keep a sheep for furnishing blood, but this is by no means necessary. Blood preserved with formalin (see p. 13) may be employed under certain conditions,¹ but freshly collected blood is better.

Corpuscle suspensions should be fairly uniform and the method described on p. 452 has been found satisfactory; it is not necessary to count the erythrocytes or estimate the hemoglobin as some workers advise, inasmuch as the color scale is prepared of each suspension.

Hemolysin.—*Antisheep hemolysin diluted with saline and titrated daily*

¹ Amer. Jour. Syph., 1919, 3, 169.

with 0.3 c.c. of 1.30 guinea-pig complement, and 0.5 c.c. of 2 per cent. sheep corpuscles.

Antisheep hemolysin is easily prepared by the immunization of rabbits, five intravenous injections of 5 c.c. of 10 per cent. suspensions of washed sheep blood every five days being a satisfactory method.¹ The animal should be bled seven to nine days after the last injection and the serum preserved by adding an equal part of best grade neutral glycerin.

Most serologists employing an antisheep hemolytic system have reported that the hemolysin requires only an occasional titration; in this technic I have not found this to be the case, owing to variation in the hemolytic activity of complement, variation in the resistance of sheep corpuscles to hemolysis and variation in guinea-pig sera. For these reasons I have found it necessary to titrate the hemolysin each time the complement-fixation tests are conducted using the same complement and corpuscles to be employed in the main tests.²

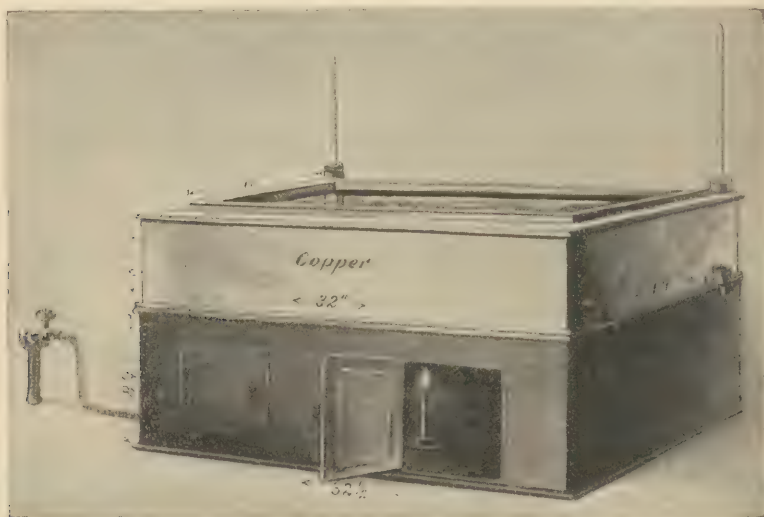


FIG. 139.—A LARGE WATER-BATH USED BY THE AUTHOR FOR THE SECONDARY INCUBATION IN COMPLEMENT-FIXATION TESTS. Size is indicated. (Amer. Jour. Syphilis, 1922, 6, 92.)

Complement.—*Dilution (1 : 30) of the mixed sera of several healthy guinea-pigs.*

The complement serum for the fixation test must be: (1) Highly sensitive to fixation by antibody and antigen; (2) possess a high degree of hemolytic activity for the erythrocytes of the indicator antigen, and (3) be free, or largely so, of agglutinins and hemolysins for the cells of the indicator antigen. Studies of the complements of different animals have shown that guinea-pig serum is best, and a mixture of the sera of three or more pigs should be used as described on p. 447.

Dilute 0.2 c.c. of serum with 5.8 c.c. of saline solution (1 : 30); this is sufficient for the hemolysin and complement titrations (these require a total of 5.7 c.c.). The balance of serum should be placed in the refrigerator and diluted later (described under complement titration) for the main tests.

¹ Amer. Jour. Syph., 1920, 4, 484.

² Ibid., 1920, 4, 616.

Titration of Hemolysin.—1. Arrange a series of ten test-tubes and place 0.5 c.c. of varying dilutions of hemolysin in each tube respectively.

2. Ordinarily a range of dilutions from 1 : 1000 to 1 : 16,000 is sufficient, but depending upon the hemolytic activity of the complement and resistance of the corpuscles higher or lower dilutions may be required. A 1 : 100 dilution preserved with phenol against bacterial contamination may be prepared as follows and kept in a refrigerator for several weeks, from which the higher dilutions are prepared as needed:

Glycerolized serum, 2.0 c.c.

Saline solution, 94.0 c.c.

Five per cent. phenol solution, 4.0 c.c.

3. The dilutions are prepared as follows in a separate set of large test-tubes:

0.2 c.c. of 1 : 100 + 1.8 c.c. saline = 1 : 1,000.

0.2 c.c. of 1 : 100 + 3.8 c.c. saline = 1 : 2,000.

0.2 c.c. of 1 : 100 + 5.8 c.c. saline = 1 : 3,000.

0.2 c.c. of 1 : 100 + 7.8 c.c. saline = 1 : 4,000.

0.2 c.c. of 1 : 100 + 9.8 c.c. saline = 1 : 5,000.

0.5 c.c. of 1 : 3000 + 0.5 c.c. saline = 1 : 6,000.

0.5 c.c. of 1 : 4000 + 0.5 c.c. saline = 1 : 8,000.

0.5 c.c. of 1 : 5000 + 0.5 c.c. saline = 1 : 10,000.

0.5 c.c. of 1 : 6000 + 0.5 c.c. saline = 1 : 12,000.

0.5 c.c. of 1 : 8000 + 0.5 c.c. saline = 1 : 16,000.

Mix contents of each tube very thoroughly.

4. To each tube, carrying 0.5 c.c. of these various dilutions of hemolysin, add 0.3 c.c. of 1 : 30 dilution of the same complement and 0.5 c.c. of a 2 per cent. suspension of the same corpuscles as used in the complement-fixation tests; add 1.7 c.c. saline to each tube to make the total volume in each 3 c.c.

5. Mix the contents of each tube and place in the water-bath at 38° C. for one hour; *the unit is the highest dilution of hemolysin showing just complete hemolysis*. Two units are employed in the titration of complement and antigen and in the complement-fixation tests.

The following table shows the ensemble and results of a titration:

TITRATION OF HEMOLYSIN

TUBE.	HEMOLYSIN.	COMPLEMENT, 1: 30.	CORPUSCLES, 2 PER CENT.	SALINE.	AFTER WATER-BATH INCUBATION FOR ONE HOUR.
1....	0.5 c.c. 1 : 1,000	0.3 c.c.	0.5 c.c.	1.7 c.c.	Complete hemolysis.
2....	0.5 c.c. 1 : 2,000	"	"	"	Complete hemolysis.
3....	0.5 c.c. 1 : 3,000	"	"	"	Complete hemolysis.
4....	0.5 c.c. 1 : 4,000	"	"	"	Complete hemolysis.
5....	0.5 c.c. 1 : 5,000	"	"	"	Complete hemolysis.
6....	0.5 c.c. 1 : 6,000	"	"	"	Complete hemolysis; unit.
7....	0.5 c.c. 1 : 8,000	"	"	"	Marked hemolysis.
8....	0.5 c.c. 1 : 10,000	"	"	"	Marked hemolysis.
9....	0.5 c.c. 1 : 12,000	"	"	"	Slight hemolysis.
10....	0.5 c.c. 1 : 16,000	"	"	"	No hemolysis.

In the above titration the unit was 0.5 c.c. of 1 : 6000; 2 units were contained in 0.5 c.c. of 1 : 3000, etc.

6. Sufficient hemolysin is now prepared so that each 2 units are contained in 0.5 c.c.

Titration of Complement.—1. Arrange ten test-tubes and place 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 c.c. of 1 : 30 complement in each respectively; the tenth tube serves as a corpuscle control for this and the hemolysin titration.

2. Into each of the first nine tubes place 10 units of antigen so diluted that this amount is contained in 0.5 c.c.

3. Place sufficient saline solution in each tube to make the total volume about 2 c.c.

4. Mix contents of each tube and place in a water-bath at 38° C. for one hour.

5. Add 0.5 c.c. hemolysin (2 units) and 0.5 c.c. corpuscle suspension (2 per cent.) to each tube; mix and re-incubate one hour.

6. Ordinarily the smallest amount of 1 : 30 complement giving complete hemolysis is taken as the unit, but *experience has shown that with the method of primary incubation employed in the complement-fixation tests, namely, fifteen to eighteen hours at 6° to 8° C., this is insufficient; in this test the unit is taken as the amount of complement in the next higher tube.* For example, if hemolysis is just complete with 0.25 c.c. the unit is taken as 0.3 c.c. and double this amount for the antigen titrations and complement-fixation tests. For convenience I have designated this amount as *two full units of complement*.¹

The following table shows the ensemble, the results of a titration, and the method of reading:

TITRATION OF COMPLEMENT

TUBE.	COMPLEMENT.	ANTIGEN (10 UNITS).	SALINE.	HEMOLYSIN (2 UNITS).	CORPUSCLES (2 PER CENT.).	AFTER WATER-BATH INCUBATION FOR ONE HOUR.
1....	0.1 c.c.	0.5 c.c.	1.4 c.c.	0.5 c.c.	0.5 c.c.	No hemolysis.
2....	0.15 c.c.	"	1.4 c.c.	"	"	Slight hemolysis.
3....	0.2 c.c.	"	1.3 c.c.	"	"	Marked hemolysis.
4....	0.25 c.c.	"	1.3 c.c.	"	"	Complete hemolysis; the exact unit.
5....	0.3 c.c.	"	1.2 c.c.	"	"	Complete hemolysis the full unit.
6....	0.35 c.c.	"	1.2 c.c.	"	"	Complete hemolysis.
7....	0.4 c.c.	"	1.1 c.c.	"	"	Complete hemolysis.
8....	0.45 c.c.	"	1.1 c.c.	"	"	Complete hemolysis.
9....	0.5 c.c.	"	1.0 c.c.	"	"	Complete hemolysis.
10....	2.0 c.c.	"	"	No hemolysis.

In practice, the hemolysin titration may be placed in the water-bath at the same time as the complement titration; at the end of the first incubation of the complement titration the unit of hemolysin is available and 2 units added to all tubes of the complement titration, etc.

7. Each 2 full units of complement are diluted with sufficient solution to make 1 c.c., called the dose of complement: for example, if 0.3 c.c. of 1 : 30 complement is the full unit, the dose is 0.6 c.c.

¹ Experience has shown that in this test the reactions are unsatisfactory if less than 0.4 c.c. of 1 : 30 complement is employed; occasionally hyperactive sera yield a unit with 0.1 c.c. of 1 : 30, but when this occurs it is necessary to use 0.4 c.c. for the dose of complement.

A convenient scheme for diluting the dose of complement to 1 c.c. is as follows: Divide 30 by the dose = the dilution to employ in amount of 1 c.c. For example:

Exact unit = 0.25 c.c. of 1 : 30 dilution.

Full unit = 0.3 c.c. of 1 : 30 dilution.

Dose = 0.6 c.c. of 1 : 30 dilution.

$$\frac{30}{0.6} = 1 : 50.$$

If 75 doses of complement were to be provided (sufficient for testing 12 sera or spinal fluids) this would require 75 c.c. of 1 : 50 prepared by diluting 1.5 c.c. guinea-pig serum with 73.5 c.c. of saline solution.

Titration of Antigen.—The antigen employed in the complement-fixation test for syphilis introduces the most important single factor of variation; the adoption of a certain kind of antigen fulfilling certain requirements is the most important factor in relation to standardization of technic.

In my opinion an antigen should be an alcoholic extract of a fresh tissue and preferably heart-muscle reinforced with 0.2 per cent. cholesterin¹; this amount of cholesterin "stabilizes" the extract and greatly increases antigenic activity with very slight or no increase of anticomplementary activity and without increasing the chances for non-specific positive reactions with heated normal human sera. A superior antigen in my experience is that previously described on p. 458, in which a mixture of dried powder of several heart-muscles are used. This powder is first extracted with ether, then with alcohol and again with alcohol; the second alcohol constitutes the base of the antigen which is reinforced with 0.2 per cent. cholesterin and all the acetone-insoluble lipoids recovered from the ether and primary alcoholic extracts.

Whatever antigen is employed it should be used in a dose of 10 antigenic units, and this amount should be at least six times less than the anticomplementary and hemolytic units. A plan for establishing a uniform unit of antigen for use in a standardized test has been developed.²

Antigen should be carefully preserved³ and titrated at least once a month unless it shows evidences of losing in antigenic activity or acquiring increased anticomplementary activity.

*Antigen should be diluted by placing the required amount of physiologic saline in a test-tube or Erlenmeyer flask and adding the required amount of extract drop by drop or in amounts of 0.1 c.c. and shaking by rotating after each addition.*⁴

In a series of ten test-tubes prepare the following dilutions of antigen:

1.0 c.c. antigen to 3.0 c.c. saline	= 1 : 4.
1.0 c.c. antigen to 4.0 c.c. saline	= 1 : 5.
0.5 c.c. antigen to 2.5 c.c. saline	= 1 : 6.
1.5 c.c. antigen 1 : 4 to 1.5 c.c. saline	= 1 : 8.
2.0 c.c. antigen 1 : 5 to 2.0 c.c. saline	= 1 : 10.
1.5 c.c. antigen 1 : 6 to 1.5 c.c. saline	= 1 : 12.
1.5 c.c. antigen 1 : 8 to 1.5 c.c. saline	= 1 : 16.
1.0 c.c. antigen 1 : 10 to 1.0 c.c. saline	= 1 : 20.
1.0 c.c. antigen 1 : 12 to 1.0 c.c. saline	= 1 : 24.
1.0 c.c. antigen 1 : 16 to 1.0 c.c. saline	= 1 : 32.

¹ Amer. Jour. Syph., 1922, 6, 74, 289.

² Ibid., 1922, 6, 651.

³ Ibid., 1922, 6, 319.

⁴ Ibid., 1922, 6, 461.

Hemolytic Titration.—1. In a series of ten regulation test-tubes place 0.5 c.c. of the above dilutions of antigen respectively.

2. To each tube add 0.5 c.c. of a 1 : 10 dilution of normal human serum previously heated for fifteen minutes at 55° C. and 1.5 c.c. of saline solution.

3. Mix the contents of each tube and place in a refrigerator at 6° to 8° C. for fifteen to eighteen hours.

4. Add 0.5 c.c. of 2 per cent. corpuscle suspension to each tube; mix and place in a water-bath at 38° C. for one hour.

5. Allow tubes to stand several hours in a refrigerator and read the results, *the smallest amount of antigen just beginning to produce hemolysis is the hemolytic unit.*

The following table shows the ensemble, the results of a titration, and the method of reading.

HEMOLYTIC TITRATION OF ANTIGEN

TUBE.	ANTIGEN, 0.5 C.C.	HEATED HUMAN SERUM, ¹ 1 : 10.	SALINE.	to for fifteen to eighteen hours.	CORPUS- CLES, 2 PER CENT.	WATER-BATH ONE HOUR.
1....	1 : 4	0.5 c.c.	1.5 c.c.	Refrigerator 6-8° C. for fifteen to eighteen hours.	0.5 c.c.	Marked hemolysis.
2....	1 : 5	"	"		"	Slight hemolysis; unit.
3....	1 : 6	"	"		"	No hemolysis.
4....	1 : 8	"	"		"	No hemolysis.
5....	1 : 10	"	"		"	No hemolysis.
6....	1 : 12	"	"		"	No hemolysis.
7....	1 : 16	"	"		"	No hemolysis.
8....	1 : 20	"	"		"	No hemolysis.
9....	1 : 24	"	"		"	No hemolysis.
10....	1 : 32	"	"		"	No hemolysis.

Anticomplementary Titration.—1. In the first ten tubes of a second series of twelve test-tubes place 0.5 c.c. of the above dilutions of antigen respectively.

2. To each of the first eleven tubes add 0.5 c.c. of a 1 : 10 dilution of normal human serum previously heated at 55° C. for fifteen minutes.

3. Add 1 c.c. of diluted complement (carrying 2 full units) to each of the twelve tubes.

4. To the eleventh tube add 0.5 c.c., and to the twelfth tube, 1 c.c. saline and place in a refrigerator at 6° to 8° C. for fifteen to eighteen hours.

5. Mix all tubes.

6. Add 0.5 c.c. hemolysin (2 units), and 0.5 c.c. of the 2 per cent. suspension of corpuscles to each tube; mix, and place in a water-bath at 38° C. for one hour. Place the tubes in a refrigerator for a few hours and read the results.

7. The *anticomplementary unit* is the smallest amount of antigen producing some inhibition of hemolysis. The eleventh tube is the serum control; the twelfth tube is the hemolytic system control and both should show complete hemolysis.

The following table shows the ensemble, the results of a titration, and method of reading:

¹ May be omitted, in which case 2 c.c. saline are added to each tube instead of 1.5 c.c.

ANTICOMPLEMENTARY TITRATION OF ANTIGEN

TUBE.	ANTIGEN.	HEATED HUMAN SERUM ¹ 1 : 10.	COMPLEMENT. (2 FULL UNITS)	HEMOLYSIN (2 UNITS).	CORPUSCLES.	WATER-BATH ONE HOUR.
1....	1 : 4	0.5 c.c.	1.0 c.c.	0.5 c.c.	0.5 c.c.	Slight hemolysis. ²
2....	1 : 5	"	"	"	"	Complete inhibition of hemolysis.
3....	1 : 6	"	"	"	"	Marked inhibition of hemolysis.
4....	1 : 8	"	"	"	"	<i>Slight inhibition of hemolysis; unit.</i>
5....	1 : 10	"	"	"	"	Complete hemolysis.
6....	1 : 12	"	"	"	"	Complete hemolysis.
7....	1 : 16	"	"	"	"	Complete hemolysis.
8....	1 : 20	"	"	"	"	Complete hemolysis.
9....	1 : 24	"	"	"	"	Complete hemolysis.
10....	1 : 32	"	"	"	"	Complete hemolysis.
11....	0.5 saline.	"	"	"	"	Complete hemolysis.
12....	1.0 saline.	"	"	"	"	Complete hemolysis.

Refrigerator 6-8° C. for fifteen to eighteen hours.

Antigenic Titrations.—1. In a series of ten test-tubes prepare the following dilutions of antigen starting with the remainder of the 1 : 10 dilution prepared above for the hemolytic and anticomplementary titrations:

- 0.1 c.c. antigen 1 : 10 to 2.9 c.c. saline = 1 : 300.
- 0.1 c.c. antigen 1 : 10 to 3.9 c.c. saline = 1 : 400.
- 0.1 c.c. antigen 1 : 10 to 4.9 c.c. saline = 1 : 500.
- 1.0 c.c. antigen 1 : 300 to 1.0 c.c. saline = 1 : 600.
- 1.0 c.c. antigen 1 : 400 to 1.0 c.c. saline = 1 : 800.
- 1.0 c.c. antigen 1 : 500 to 1.0 c.c. saline = 1 : 1000.
- 1.0 c.c. antigen 1 : 600 to 1.0 c.c. saline = 1 : 1200.
- 1.0 c.c. antigen 1 : 800 to 1.0 c.c. saline = 1 : 1600.
- 1.0 c.c. antigen 1 : 1000 to 1.0 c.c. saline = 1 : 2000.
- 1.0 c.c. antigen 1 : 1200 to 1.0 c.c. saline = 1 : 2400.

2. Arrange a series of twelve regulation test-tubes and place 0.5 c.c. of the above dilutions of antigen into the first ten tubes respectively.

3. In each of the first eleven tubes place 0.5 c.c. of a 1 : 10 dilution of a *mixture of equal parts of four or more freshly collected syphilitic and Wassermann positive sera previously heated at 55° C. for at least fifteen minutes.*

4. In each tube place 1 c.c. of diluted complement (carrying 2 full units).

5. To the eleventh tube add 0.5 c.c. and to the twelfth tube 1 c.c. of saline solution.

6. Mix contents of all tubes and place in a refrigerator at 6° to 8° C. for fifteen to eighteen hours.

7. Add 0.5 c.c. hemolysin (2 units) and 0.5 c.c. of the 2 per cent. corpuscle suspension to all tubes; mix, and place in a water-bath at 38° C. for one hour. Place tubes in a refrigerator for a few hours and read the results.

8. The *antigenic unit* is the highest dilution of antigen giving complete inhibition of hemolysis. The eleventh tube is the serum control, and the twelfth tube the hemolytic system control; both should show complete hemolysis.

¹ May be omitted and 0.5 c.c. saline added instead.

² Due to the hemolytic activity of the antigen.

The following table shows the ensemble, the results of a titration, and method of reading:

ANTIGENIC TITRATION OF ANTIGEN

TUBE.	ANTIGEN, 0.5 C.C.	HEATED SYPHI- LITIC SERA, 1:10.	COMPLE- MENT (2 FULL UNITS).	hours.	HEMOL- YSIN (2 UNITS).	CORPUS- CLES, 2 PER CENT.	WATER-BATH ONE HOUR
1....	1:300	0.5 c.c.	1.0 c.c.	Refrigerator at 6-8° C. for fifteen to eighteen hours.	0.5 c.c.	0.5 c.c.	Complete inhibition of hemolysis.
2....	1:400	"	"		"	"	Complete inhibition of hemolysis.
3....	1:500	"	"		"	"	Complete inhibition of hemolysis.
4....	1:600	"	"		"	"	Complete inhibition of hemolysis.
5....	1:800	"	"		"	"	Complete inhibition of hemolysis.
6....	1:1000	"	"		"	"	Complete inhibition of hemolysis.
7....	1:1200	"	"		"	"	Complete inhibition of hemolysis.
8....	1:1600	"	"		"	"	Complete inhibition of hemolysis.
9....	1:2000	"	"		"	"	Complete inhibition of hemolysis.
10....	1:2400	"	"		"	"	Complete inhibition of hemolysis.
11....	0.5 saline.	"	"		"	"	Complete inhibition of hemolysis (unit).
12....	1.0 saline.	"	"		"	"	Marked inhibition of hemolysis.
							Slight inhibition of hemolysis.
							Complete hemolysis.

9. Ten antigenic units are used in conducting the complement-fixation tests. For example, if the unit is 0.5 c.c. of a 1:1600 dilution, as shown in the above table, the dose of 10 units would be contained in 0.5 c.c. of 1:160 dilution.

The Quantitative Complement-fixation Test.—1. Sera should be properly prepared and heated in a water-bath at 55° C. for fifteen minutes; spinal fluids are used unheated. The tests should be set up in the following order: Serum first, followed by antigen, and lastly by complement.

2. For each *serum* arrange six regulation test-tubes and place saline solution in the first five in the following amounts respectively: 1.2, 0.5, 0.5, 2.0, and 0.5 c.c.

Into the first tube place 0.3 c.c. serum and mix; transfer 0.5 c.c. to tubes Nos. 2 and 6.

Mix No. 2 and transfer 0.5 c.c. to tube No. 3.

Mix No. 3 and transfer 0.5 c.c. to tube No. 4.

Mix No. 4 and transfer 0.5 c.c. to tube No. 5; discard 1.5 c.c.

Mix No. 5 and discard 0.5 c.c.

Each tube now contains 0.5 c.c. carrying 0.1, 0.05, 0.025, 0.005, 0.0025, and 0.1 c.c. (serum control), as employed by Detweiler¹ in his quantitative test.

3. For each spinal fluid arrange six regulation tubes and place 0.5 c.c. saline in Nos. 2, 3, 4, 5, and 6.

Into the first, second, and sixth tubes place 0.5 c.c. spinal fluid; mix No. 2 and transfer 0.5 c.c. to No. 3; mix No. 3 and transfer 0.5 c.c. to No. 4; mix No. 4 and transfer 0.5 c.c. to No. 5; mix No. 5 and discard 0.5 c.c.

¹ *Canad. Med. Assn. Jour.*, January, 1916.

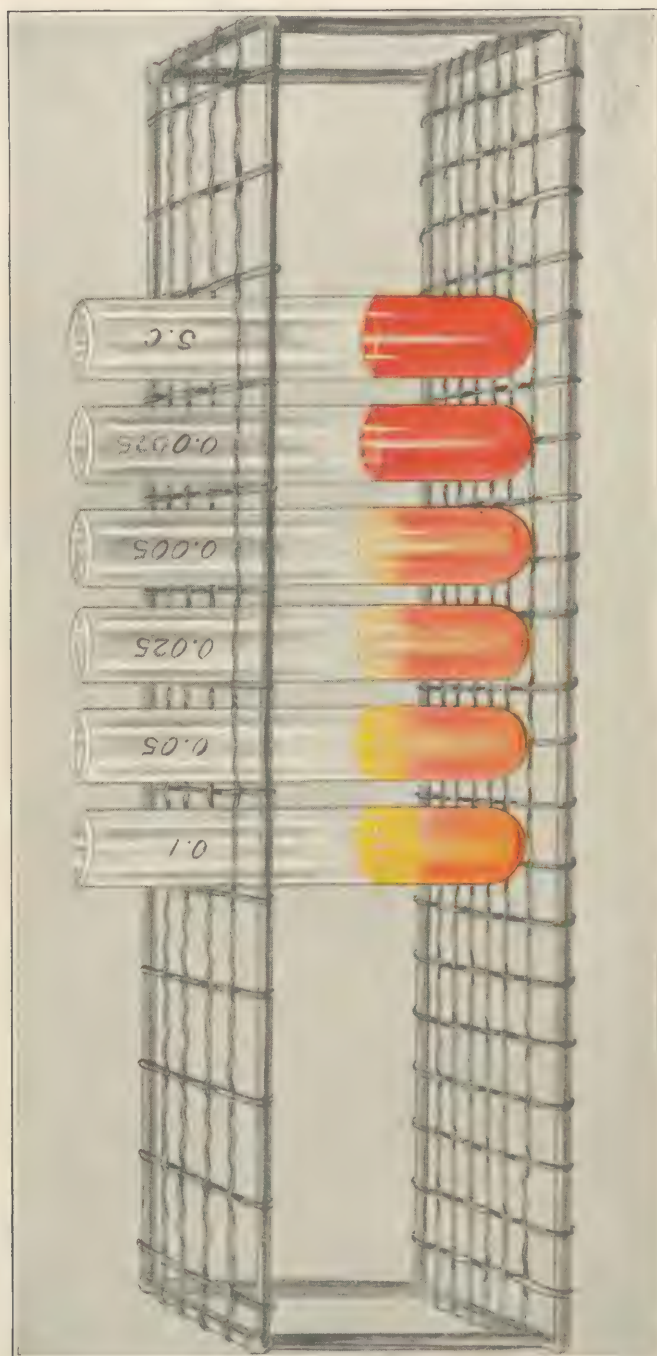


FIG. 140.—A POSITIVE REACTION WITH THE NEW COMPLEMENT-FIXATION TECHNIC.
Shows test-tubes slightly reduced in size, the volume in each and depth of color. The reaction is 4421 (very strongly positive).
(*Amer. Jour. Syphilis*, 1922, 6, 92.)

Each tube now contains 0.5 c.c. carrying 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.5 c.c. (control).

4. Into the first five tubes of each set add 0.5 c.c. antigen dilution (carrying ten antigenic units).

5. After an interval of five to thirty minutes add 1.0 c.c. of complement to each tube (carrying two full units).

6. Include the following controls: (a) *Antigen control* tube carrying 0.5 c.c. of the diluted antigen, 1 c.c. of the diluted complement, and 0.5 c.c. of saline solution; (b) *hemolytic system control* carrying 1 c.c. of the diluted complement and 1 c.c. of saline solution; (c) *corpuscle control* carrying 2.5 c.c. of saline solution; (d) *positive* and *negative* serum controls should be included using syphilitic and normal sera respectively, set up in the various amounts as described above.

7. Prepare a reading scale as follows:

(a) Heat 6 c.c. of the diluted complement in a water-bath at 55° C. for fifteen minutes.

(b) Prepare a solution of hemoglobin by dissolving 2 c.c. of the 2 per cent. corpuscle suspension in 4 c.c. of plain water.

(c) Arrange a series of five regulation test-tubes numbered 1 to 5 and place in each: 0.5 c.c. of the diluted antigen and 1 c.c. of the heated diluted complement.

(d) Add hemoglobin solution to the first four tubes as follows: 1.5, 1.13, 0.75, and 0.38 c.c.

(e) Add 0.24 c.c. physiologic saline solution to No. 2; 0.5 c.c. to No. 3; 0.74 c.c. to No. 4, and 1 c.c. to No. 5.

Corpuscles are added the following day.

8. Mix the contents of all tubes gently, but thoroughly, and place in a refrigerator at 6° to 8° C. for fifteen to eighteen hours.

9. Warm the tubes in a water-bath at 30° C. for *five to fifteen minutes, but not longer*¹ and add 0.5 c.c. hemolysin (carrying two units) to all tubes except the corpuscle control; thoroughly mix the 2 per cent. corpuscle suspension (which has been carried over in a refrigerator from the previous day) and add 0.5 c.c. to all tubes.

THE QUANTITATIVE COMPLEMENT-FIXATION TEST FOR SYPHILIS (FIG. 140)

TUBE.	PATIENT'S SERUM IN 0.5 C.C. ²	ANTIGEN, 10 UNITS.		COMPLEMENT (2 FULL UNITS).	for C. at 6-8° incubation at fifteen to eighteen hours.	HEMOLYSIN (2 UNITS).	CORPUSCLES, 2 PER CENT.	
1.....	0.1 c.c.	0.5 c.c.	Wait five to thirty minutes.	1.0 c.c.	Primary incubation at 6-8° C. for fifteen to eighteen hours.	0.5 c.c.	0.5 c.c.	Secondary incubation one hour at 38° C. Read one to three hours later with scale.
2.....	0.05 c.c.	"		"		"	"	
3.....	0.025 c.c.	"		"		"	"	
4.....	0.005 c.c.	"		"		"	"	
5.....	0.0025 c.c.	"		"		"	"	
6.....	0.1 c.c. control.		"		"	"	
7.....	Control antigen.	0.5 c.c.		"		"	"	
8.....	Control hemolytic.		2.5 c.c.		"	"	
9.....	Control. corpuscle.		saline.		"	

¹ This preliminary warming may be omitted; if more than fifteen minutes are used some non-specific reactions may occur.

² Spinal fluid doses: 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.5 c.c. (control).

10. Add corpuscle suspension to the tubes of the reading scale as follows: 0.13 c.c. to No. 2, 0.25 c.c. to No. 3, 0.38 c.c. to No. 4, and 0.5 c.c. to No. 5.

11. Mix the contents of all tubes gently but thoroughly, and place in a water-bath at 38° C. for one hour (the water must reach above the level of the contents in the tubes).

12. Place all tubes in a refrigerator for one to three hours to permit the partial settling of non-hemolyzed corpuscles; the degree of inhibition hemolysis is then read off and recorded for each tube with the aid of the reading scale as —, + (1), ++ (2), +++ (3), or ++++ (4). All serum controls, antigen, and hemolytic controls should show complete hemolysis.

13. Tube No. 1 of the color scale shows a — reaction; tube No. 2 shows a +; tube No. 3 shows a ++, tube No. 4 shows a +++, and tube No. 5 shows a ++++ reaction.

Experience has shown that the reactions may be interpreted as follows:

Very strongly positive if there is partial or complete fixation of complement in the first four or five tubes. Occasionally a serum will show less fixation of complement in the first tube carrying 0.1 c.c. serum than the second tube carrying 0.05 c.c. as a ++, ++++, +++, + and — (control) reaction (recorded as 2, 4, 3, 1, —). It may be assumed that this is due to the presence of natural antisheep hemolysin, but I have seen the phenomenon occur with hemolysin-free sera and believe that it is due to the presence of other serum constituents in this relatively large amount of serum interfering with the fixation of complement by antigen and syphilis antibody. So far I have not seen this occur with spinal fluids.

Strongly positive if there is a partial or complete fixation of complement in the first three tubes.

Moderately positive if there is partial or complete fixation of complement in the first two tubes.

Weakly positive if there is partial or complete fixation of complement in the first tube.

Negative when all tubes show complete hemolysis.

The following results of tests with a syphilitic serum and spinal fluid shows the method of recording and reporting:

Quantitative Reaction.—Moderately positive (42 — — —).

Serum 0.1	c.c. = ++++.
Serum 0.05	c.c. = ++.
Serum 0.025	c.c. = —.
Serum 0.005	c.c. = —.
Serum 0.0025	c.c. = —.
Serum 0.1 (control)	c.c. = —.

Quantitative Reaction.—Very strongly positive (44442).

Spinal fluid 0.5	c.c. = ++++.
Spinal fluid 0.25	c.c. = ++++.
Spinal fluid 0.125	c.c. = ++++.
Spinal fluid 0.0625	c.c. = ++++.
Spinal fluid 0.03125	c.c. = ++.
Spinal fluid 0.5 (control)	c.c. = —.

The Qualitative Complement-fixation Test.—The qualitative test is conducted in the same manner as the quantitative test except that two tubes, instead of six, are employed; otherwise the technic is the same and may be described more briefly:

1. For each *serum* arrange two regulation test-tubes and place 0.8 c.c.

saline and 0.2 c.c. serum in the first; mix the contents and transfer 0.5 c.c. to the second tube (control).

2. For each *spinal fluid* arrange two regulation test-tubes and place 0.5 c.c. spinal fluid in each.

3. Add 10 units of antigen (0.5 c.c. dilution) to the first tube and 0.5 c.c. of saline solution to the second tube or control tube, of each series.

4. After waiting five to thirty minutes add 1 c.c. of diluted complement (carrying two full units) to both tubes of each set.

5. Include corpuscles, hemolytic system, and antigen controls as previously described; also a syphilitic serum and normal serum for a positive and negative reaction, as described above.

6. Set up first part of the reading scale as described with the quantitative test.

7. Mix the contents of all tubes gently but thoroughly, and place all tubes in a refrigerator at 6° to 8° C. for fifteen to eighteen hours.

8. Warm the tubes in a water-bath at 38° C. for *five to fifteen minutes (not longer)*, add 0.5 c.c. hemolysin (2 units) to all tubes except the corpuscle control. Gently but thoroughly mix the 2 per cent. corpuscle suspension carried over in the refrigerator from the previous day and add 0.5 c.c. to each tube.

9. Finish the reading scale by adding corpuscles as previously described.

10. Mix the contents of all tubes gently but thoroughly, and incubate in a water-bath at 38° C. for one hour.

11. Place the tubes in a refrigerator for one to three hours to permit the partial settling of non-hemolyzed corpuscles and read the results with the aid of the scale. All serum, the antigen, and hemolytic controls should show complete hemolysis; the corpuscle control should show no hemolysis.

THE QUALITATIVE COMPLEMENT-FIXATION TEST FOR SYPHILIS

TUBE.	PATIENT'S SERUM IN 0.5 C.C.	ANTIGEN (10 UNITS).		COMPLEMENT (2 FULL UNITS).		HEMOLYSIN (2 UNITS).	CORPUSCLES, 2 PER CENT.	
1.....	0.1 c.c.	0.5 c.c.	Wait five to ten minutes.	1.0 c.c.	Primary incubation fifteen to eighteen hours at 6-8° C.	0.5 c.c.	0.5 c.c.	Secondary incubation one hour at 38° C. Read one to three hours later with aid of scale.
2.....	0.1 c.c. (control).		"		"	"	
3.....	Antigen (control).	0.5 c.c.		"		"	"	
4.....	Hemolytic (control).		"		"	"	
5.....	Corpuscle (control).		2.5 c.c.		"	

12. The results are read with the aid of the reading scale as previously described and recorded according to the + + + +, + + +, + +, +, and - method of recording complement fixation in the first tube of each set: + + + + = strongly positive (100 per cent. inhibition of hemolysis), + + + = moderately positive (about 75 per cent. inhibition of hemolysis), + + = weakly positive (about 50 per cent. inhibition of hemolysis), and + = very weakly positive (about 25 per cent. inhibition of hemolysis), - = negative (complete hemolysis).

The Relative Value of the Quantitative and Qualitative Tests.—Experience has shown that the quantitative test is more satisfactory than the qualitative test. It is true that more test-tubes and materials are required,

but these and the slightly greater time required for conducting the tests are more than compensated for by the results. With about $\frac{1}{2}$ per cent. of sera the first dose of 0.1 c.c. may yield a weaker reaction than the smaller amounts, as 0.05 and 0.025 c.c.; indeed, with weakly positive sera the 0.1 c.c. amount may yield a falsely negative reaction, whereas true positive reactions occur with the 0.05 c.c. amount. As previously stated, Detweiler has noticed this phenomenon in his quantitative test and believes that it is due to the influence of natural antishoop hemoglobin, but I have observed the reaction with hemolysin-free sera and have never seen it with spinal fluids containing some hemolysin. In my opinion it is due to interference of complement fixation by some other serum constituent. Whatever may be the true explanation, the fact remains that it occurs and constitutes the main reason for using varying amounts of patient's serum in conducting the syphilis complement-fixation test; furthermore, the quantitative method gives a better serologic guide to treatment.

For example, a qualitative test yielding a ++++ reaction may not show any reduction in the strength of the reaction despite considerable treatment because the reagin content of the serum has not been reduced below the ++++ level; under these circumstances the physician may surmise that there has been no serologic improvement even though clinical improvement has taken place. On the other hand, the quantitative test employing smaller amounts of serum will show in a clear manner the degree of serologic improvement, as demonstrated in the following record of a case of syphilis in the secondary stage treated with neo-arsphenamin:

SERUM.	BEFORE TREATMENT.	AFTER 6 INJECTIONS.	AFTER 12 INJECTIONS.	AFTER 15 INJECTIONS.
0.1.....	++++	++++	+	—
0.05.....	++++	++	—	—
0.025.....	++++	—	—	—
0.005.....	++	—	—	—
0.0025.....	—	—	—	—

By means of this quantitative reaction it is possible to plot a curve for each case under treatment. Such curves show fluctuations similar to those observed by Vernes with his precipitation reaction, with a gradual tendency for reduction to a negative reaction.

The Quantitative and Qualitative Tests with an Antiox Hemolytic System.—In those laboratories where a sheep cannot be maintained or sheep blood regularly obtained from an abattoir, beef blood may be used with entire success and satisfaction. The technic is exactly the same as described except that beef corpuscles are used instead of sheep.

Not infrequently it is more difficult to prepare highly potent antiox hemolysin by immunization of rabbits than antishoop; under these circumstances it may be necessary to dilute the complement 1 : 20 for the hemolysin and complement titrations instead of 1 : 30 as described for the antishoop system.

When the hemolysin is of such strength as permits the use of 1 : 30 complement, the results are almost identical to those observed with the antishoop system¹; hemolysins of this strength can and should be used rather than a weak hemolytic serum requiring the use of 1 : 20 complement.

¹ Amer. Jour. Syph., 1922, 6, 667.

The Quantitative and Qualitative Tests with Antihuman and Antichicken Hemolytic Systems.—Experience has shown that *tests conducted with antisheep and antiox hemolytic systems are slightly more sensitive than tests conducted with the antihuman and antichicken systems.*¹ I believe that these differences are due to the dilution and amount of complement and not to natural hemolysins in human sera. It appears to be a fundamental principle that *the higher the dilution of complement, that is, the smaller the amount of complement serum we may use, the more sensitive are the complement-fixation reactions.* The difficulties encountered in the preparation of antihuman and antichicken hemolysins are such that it is usually necessary to use the complement diluted 1 : 5 or 1 : 10, and *these larger amounts of guinea-pig serum complement reduce the sensitiveness of reactions with the new technic probably by the introduction of serum proteins interfering with specific complement fixation.* The presence or absence of natural hemolysins in the complement serum appear to be of much less importance because the results are the same regardless of the presence or absence of these substances in the complement or patients' sera.

TECHNIC OF THE FOURTH METHOD

AUTHOR'S MODIFICATION OF THE WASSERMANN REACTION WITH VARYING AMOUNTS OF COMPLEMENT

In this method the amount of syphilis antibody in a serum is measured according to the number of hemolytic doses of complement absorbed or fixed with a constant amount of antigen. As previously stated, any organic extract used as antigen may of itself fix a certain amount of complement; a non-syphilitic serum may do the same, and a mixture of the two may fix still more, though the amounts may be relatively small. A peculiarity possessed by a syphilitic serum is that it fixes a large amount of complement when mixed with antigen; as a result the test becomes a quantitative and not a qualitative reaction. The only practical means we possess of estimating the amount of complement in a fresh serum is to ascertain the hemolytic dose, *i. e.*, to find the smallest quantity of serum that is just sufficient completely to lyse the test amount of corpuscles with the hemolysin. When this has been done, the quantity of complement used in the reaction may be expressed in terms of hemolytic doses fixed, and not in terms of the amount of fresh serum.

When properly performed according to this method, which has been modified after the technic of Browning and Mackenzie² and Thomsen,³ the syphilis reaction becomes quite delicate and accurate, but is more complicated than the other methods, and should not be attempted until one is accustomed to the simpler test and thoroughly understands the underlying principles of the syphilis reaction and knows the many sources of fallacy. The greater amount of work that it entails and the larger quantities of complement-serum and amboceptor that are required may serve as factors against its adoption as a routine method. On the other hand, the sources of error are well under control, and the test has yielded remarkably uniform results in the hands of my colleagues in their respective laboratories, working with the serums of the same persons.

1. Corpuscles.—Sheep corpuscles are washed three times with salt solution and made up in a $2\frac{1}{2}$ per cent. suspension; dose, 0.5 c.c.

¹ Amer. Jour. Syph., 1922, 6, 667.

² Ztschr. f. Immunitätsf., orig., 1909, 2, 459.

³ Ztschr. f. Immunitätsf., orig., 1910, 7, 389.

2. **Hemolysin.**—Antisheep hemolysin is titrated by placing increasing amounts of diluted serum, as 0.1 c.c., 0.15 c.c., 0.25 c.c., 0.30 c.c., and 0.35 c.c. of a 1 : 300 dilution in a series of test-tubes and adding to each tube 0.025 complement serum (= 0.5 c.c. of a 1 : 20 dilution), 0.5 c.c. of 2½ per cent. corpuscle suspension, and sufficient salt solution to make the total volume in each tube about 3 c.c. Each tube is gently shaken and incubated in the water-bath at 38° C. for one hour, when the reading is made. The unit of hemolysin is the smallest amount giving complete hemolysis. Instead of using increasing amounts of one dilution of hemolysin as above, a series of dilutions may be made in flasks, as 1 : 2000; 1 : 2500; 1 : 3000; 1 : 3500; 1 : 4000, etc., and 1 c.c. of each used in the titration.

The unit need not be determined more than once in two weeks, providing the hemolysin is kept at a low temperature.

3. **Complement.**—It is advisable to use the mixed sera of at least two or more healthy guinea-pigs. When only a small amount of complement serum is required, sufficient blood may be obtained by aspirating 2 c.c. of blood from the hearts of several large animals (see page 37). The complement serum should be clear, collected a few hours before use, and preferably from fasting animals. The mixed serum is diluted with 9 parts of normal salt solution (1 : 10) and titrated. This titration must be performed with every complement serum before the main tests are conducted. In order to allow for the anticomplementary action of the antigen the complement is titrated as suggested by Thomsen, in the presence of the dose of antigen, as determined by titration, to be used in the main tests, as follows: In each of a series of six test-tubes place the dose of antigen (as, for example, 0.1 c.c. of 1 : 20 dilution of acetone-insoluble lipoids); add the following amounts of complement 1 : 10 with an accurate pipet: 0.1 c.c., 0.2 c.c., 0.3 c.c., 0.4 c.c., 0.5 c.c., and 0.6 c.c. Add sufficient salt solution to make the total volume in each tube about 2 c.c., mix gently, and incubate in a water-bath at 38° C. for one hour, then one unit of hemolysin and 0.5 c.c. of 2½ per cent. suspension of cells are added, the tubes shaken and re-incubated in the water-bath for an hour, when the reading is made. *The unit of complement is the smallest amount producing complete hemolysis.* The results of a titration are shown in the following table and Fig. 141.

RESULTS OF COMPLEMENT TITRATION IN THE PRESENCE OF ANTIGEN

TUBE No.	ANTIGEN 1 : 20, C.C.	COMPLEMENT 1 : 10, C.C.	Add salt solution to make 2 c.c.; shake gently and incubate in water-bath at 38° C. for one hour.	HEMOLY- YSIN.	CELLS 2½ PER CENT., C.C.	Shake gently and incubate in water-bath at 38° C. for one hour.	RESULTS.
1...	0.2	0.1		1 unit.	0.5		Very weak hemolysis.
2...	0.2	0.2		1 unit.	0.5		Marked hemolysis.
3...	0.2	0.3		1 unit.	0.5		Almost complete hemolysis.
4...	0.2	0.4		1 unit.	0.5		Complete hemolysis, the unit.
5...	0.2	0.5		1 unit.	.5		Complete hemolysis.
6...	0.2	0.6		1 unit.	0.5		Complete hemolysis.

4. **Antigen.**—A large number of comparative tests with the same sera and a variety of antigens have shown that the author's new cholesterolized and lecithinized alcoholic extract of beef heart is best adapted for this reaction. As a general rule these extracts possess a marked degree of antigenic sensitiveness and are usually free of anticomplementary action except

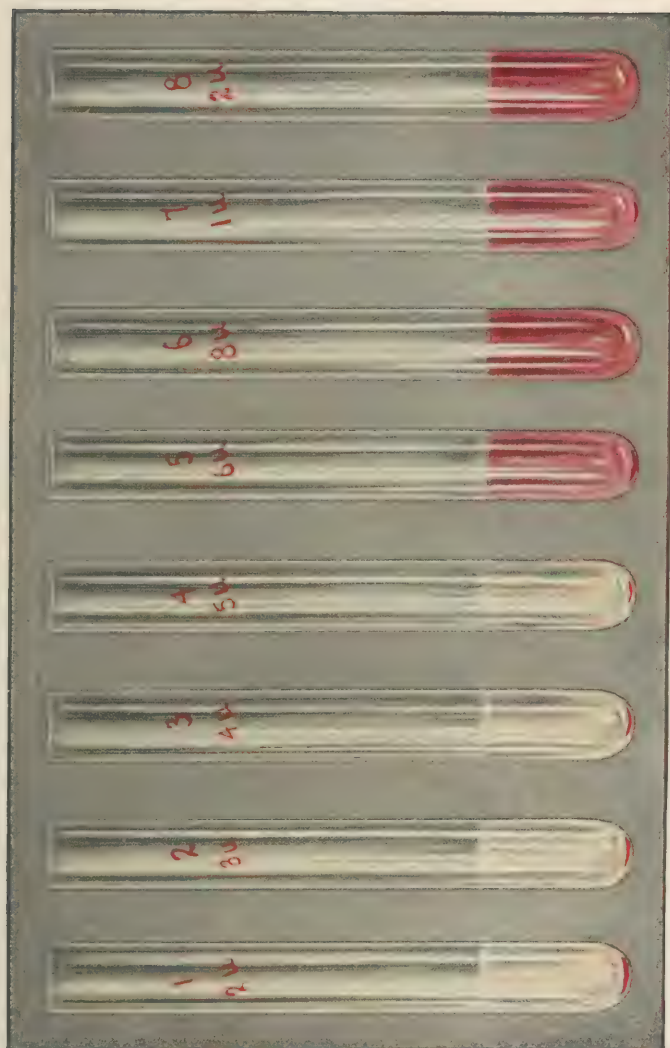


FIG 141. THE WASSERMANN REACTION AFTER THE QUANTITATIVE OR FOURTH METHOD. Shows the fixation of four units of complement. There is partial fixation with five units, but the serum alone fixed a unit (tube 7).



in very large doses. Each extract must be titrated to determine the proper dose to employ; as a general rule it is sufficient to titrate an extract once every two months providing it is carefully refrigerated and is yielding satisfactory results in complement-fixation tests.

In conducting these titrations a mixed complement serum diluted 1 : 10 is titrated in the following doses with one unit of hemolysin and 0.5 c.c. of a 2½ per cent. suspension of cells: 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.25 c.c., 0.3 c.c., and 0.4 c.c. In the *antigenic titration* the following amounts of antigen diluted 1 : 20 are placed in a series of ten test-tubes: 0.001 c.c., 0.005 c.c., 0.008 c.c., 0.01 c.c., 0.05 c.c., 0.08 c.c., 0.1 c.c., 0.2 c.c., 0.3 c.c., and 0.4 c.c.; 0.1 c.c. of fresh inactivated *syphilitic serum*, preferably from several persons mixed, plus 2 units of complement and sufficient salt solution to make 3 c.c., are added to each tube and incubated in a water-bath at 38° C. for one hour, when 1 unit of hemolysin and 0.5 c.c. of corpuscles are added, tubes shaken, and re-incubated for an hour. The reading may be made at once or after the corpuscles have settled; *the unit is the smallest amount of antigen yielding complete inhibition of hemolysis.*

In the *anticomplementary titration* the extract is diluted 1 : 20 and the following amounts placed in a series of ten test-tubes with 0.1 c.c. of fresh inactivated *normal serum* and 2 units of complement: 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 1.2, 1.5, and 2.0 c.c. The titration is conducted in the same manner, and *the anticomplementary unit is the smallest amount of extract producing inhibition of hemolysis.*

Hemolytic and serum controls on both the syphilitic and normal serum should be included and show complete hemolysis.

A satisfactory extract is one in which the antigenic unit is at least ten times less than the anticomplementary unit, and in conducting the Wassermann reaction 2 units of antigen are employed as the dose.

5. **Fluid to be Tested.**—Serum is heated at 56° C. for half an hour and used in dose of 0.1 c.c. Cerebrospinal fluid should be fresh and is used unheated in dose of 1 c.c.

6. **The Test.**—For each serum eight test-tubes are arranged in a row. One is labeled with the patient's name and all are numbered. Into each is placed 0.1 c.c. of the patient's serum. Into each of the first six tubes are placed the dose of antigen and 2, 3, 4, 5, 6, and 8 units of complement respectively. The last two tubes are the serum controls, to determine the amount of complement fixed by serum alone, and receive 1 and 2 units of complement respectively. Sufficient salt solution is added to each tube to bring the total volume up to 3 c.c., and all are incubated in the water-bath at 38° C. for an hour. One unit of hemolysin and 0.5 c.c. of corpuscle suspension are now added to each tube and re-incubated in the water-bath for one hour, when the readings are made. Sharper readings may be made after the corpuscles have settled either by centrifuging or standing the tubes in the refrigerator overnight.

An antioxy or antihuman hemolytic system may be employed if sufficiently potent hemolysins are at hand.

Controls.—1. The anticomplementary action of each serum is controlled in the last two tubes of each series; unless a serum is markedly anticomplementary, the use of 1 and 2 units of complement is sufficient.

2. A known positive and negative serum may be included.

3. A hemolytic control is set up with 1 unit of complement and antigen; after the primary incubation 1 unit of hemolysin and the cells are added. This tube is a control on the unit of complement and should show complete hemolysis.

4. A corpuscle control may be included, containing 0.5 c.c. of corpuscles and 3 c.c. of salt solution. It controls the tonicity of the salt solution and should show no hemolysis.

Reading the Results.—The controls are first inspected. The corpuscle control should show no hemolysis and the hemolytic control be just hemolyzed. The last two tubes of each series are the serum control tubes, and the first tube containing 1 unit of complement may show incomplete hemolysis, while the second tube containing 2 units of complement shows complete hemolysis unless the serum is quite anticomplementary.

RESULTS AND METHOD OF READING THE WASSERMANN REACTION (QUANTITATIVE METHOD)

No.	DIAG- NOSIS.	RESULTS OF COMPLEMENT-FIXATION TESTS.						SERUM CONTROLS.		AMOUNT OF COMPLE- MENT ABSORBED; READINGS.
		2 Units.	3 Un ts.	4 Units.	5 Units.	6 Units.	8 Units.	1 Unit.	2 Units.	
1	Normal.	—	—	—	—	—	—	—	—	Negative.
2	Normal.	+	—	—	—	—	—	±	—	Negative.
3	Syphilis.	++++	++++	+++	+	—	—	±	—	Positive; 4 units.
4	Syphilis.	+++	++	±	—	—	—	±	—	Positive; 3 units.
5	Syphilis.	++	—	—	—	—	—	—	—	Positive; 2 units.
6	Syphilis.	++++	++++	++++	++++	++++	++++	+	—	Positive; at least 8 units.
7	Syphilis.	++++	++++	++++	++++	++++	++++	+	±	Positive; at least 8 units.
8	Syphilis.	++++	++	+	—	—	—	+	±	Positive; 2 to 3 units.

The first six tubes of each series show whether or not the reaction is positive or negative, and if positive, the amount of complement absorbed. If tube No. 7 of the serum controls shows some inhibition of hemolysis, 1 unit is subtracted from the number of units of complement absorbed in the first six tubes, and the difference represents the amount of complement absorbed by antigen and syphilitic antibody. *I regard the reaction as positive when lysis is incomplete with 2 units of complement, in addition to the amount absorbed by the serum alone.* More strongly reacting serums will absorb from 3 to 8 units of complement, and not infrequently more; these sera may be retested with 8, 10, 12, etc., units of complement, but the employment of these large doses routinely is not justified because of the large amount of complement used, and the complement-fixing power of the majority of sera are readily measured with 2 to 8 units. The method of reading is best shown in the preceding table and Fig. 141.

Cases of syphilis progressing favorably with the administration of specific remedies show less and less complement fixation until a negative reaction is secured. A large number of comparative tests employing the original Wassermann and this reaction with the same antigen have shown that a serum yielding a ++++ result in the original Wassermann reaction may show anywhere from 2 to 8 units of fixation with this technic.

HYGIENIC LABORATORY METHOD

The following method is an abbreviated description of the method which has been described by Neill¹ as employed in the Hygienic Laboratory of the United States Public Health Service:

Saline Solution.—0.9 per cent. solution of pure salt in distilled water; sterilized.

Corpuscles.—Washed sheep corpuscles suspended in sufficient salt solution to restore the volume of defibrinated blood employed. For use 5 c.c.

¹ Reprint No. 483 from the Public Health Reports, August 23, 1918, 1387.

of this suspension is placed in 95 c.c. of salt solution, giving a 5 per cent. suspension of whole blood (corresponds to about a 2 per cent. suspension of washed packed cells).

Hemolysin.—Antisheep hemolysin prepared by the immunization of rabbits. Dilute 0.1 c.c. of serum with 19.9 c.c. salt solution (= 1 : 200). In a series of six test-tubes place increasing amounts as follows: 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 c.c. Add 1 c.c. of 1 : 20 dilution of the pooled sera of 5 guinea-pigs for complement and 1 c.c. of 5 per cent. suspension of sheep corpuscles. Incubate in a water-bath at 37° C. for one hour and stand in a refrigerator overnight. The smallest amount of hemolysin giving complete hemolysis is the unit; if the unit is more than 0.4 c.c. of 1 : 200 the hemolysin should be rejected. The titration should be done at least once in six weeks.

Complement.—The pooled sera of several guinea-pigs; titrated daily just before the main tests are set up.

“Estimate, in round numbers, the number of cubic centimeters of red-cell suspension needed for the day’s work: for example, 100 c.c. Multiply the unit of amboceptor by 200 and place that amount of amboceptor serum in a 100 c.c. glass-stoppered graduated cylinder. Add about 50 c.c. of 0.9 per cent. sodium chlorid solution, taking care to wash down the serum adhering to the sides of the cylinder; next add 5 c.c. of the undiluted sheep corpuscles which have been made up to the volume of the defibrinated blood. Then make up to 100 c.c. with 0.9 per cent. sodium chlorid solution. Invert fifty times to mix thoroughly. Set aside for fifteen minutes.”

Dilute complement 1 : 10 and place 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 c.c. in a series of test-tubes; add 1 c.c. of the corpuscle-hemolysin suspension and sufficient salt solution to make 4 c.c. Water-bath incubation one hour. The unit is the smallest amount of complement giving complete hemolysis.

Antigen.—Acetone-insoluble lipoids titrated in two series of test-tubes for anticomplementary and antigenic activities in the following amounts of 1 : 10 dilution: 2.0, 1.6, 1.4, 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.06, and 0.04 c.c. In the anticomplementary titration 0.2 c.c. of heated known negative serum is added to each tube; in the antigenic titration 0.2 c.c. of heated syphilitic serum. Add two units of complement to each tube and sufficient salt solution to make the total volumes about 4 c.c. Mix the contents of the tubes and place in a water-bath at 37° C. for one hour. Add 1 c.c. of the amboceptor-corpuscle suspension to each tube, mix, and re-incubate for a half hour; set aside in a refrigerator overnight. In the main tests the dose of antigen employed is several times the smallest amount giving complete inhibition of hemolysis in the titration with syphilitic serum providing this dose is not more than one-half the anticomplementary unit.

Serum.—Heated in a water-bath to 54° to 56° C. for one-half hour; dose, 0.2 c.c.

Tests.—For each serum arrange two tubes; of each place 0.2 c.c. in the front tube and 0.4 c.c. in the rear (control). Add one dose of antigen to the front tubes and two doses of complement to both tubes. Add sufficient salt solution to make the total volume 3 c.c. in each tube.

Include controls with known positive and negative sera; also an antigen control carrying two doses, a hemolytic system and corpuscle controls.

Mix well by individually agitating each tube; incubate in a water-bath at 37° C. for one hour. Add to each tube 1 c.c. of the amboceptor-sheep corpuscle suspension. Mix well and re-incubate for one-half hour. Place in a refrigerator overnight.

Readings are made as follows:

- 70 to 100 per cent. fixation = "strongly positive."
- 40 to 70 per cent. fixation = "positive."
- 20 to 40 per cent. fixation = "weakly positive."
- 0 to 20 per cent. fixation = "negative."

NOGUCHI METHOD

Among the large number of modifications of the original syphilis reaction that have been devised, that of Noguchi has proved of distinct value. In this method an antihuman hemolytic system is employed that eliminates one possible source of error due to the natural antisheep amboceptor in human serum, although the importance of this has been overemphasized and the possible influence of natural antihuman hemolysins in some serums overlooked.

Noguchi advocated the use of active serum for this test, with an antigen of acetone-insoluble lipoids. Active serum yields a more delicate reaction, but may give false or proteotropic complement fixation, especially when crude alcoholic extracts are used as antigens. I may state, however, that when a good antigen of acetone-insoluble lipoids is used, the percentage of false reactions is relatively small, being less than 2 per cent. The Noguchi test, on the other hand, may be conducted with inactivated serums when the danger of false reactions is removed, but the delicacy of the test is likewise diminished, so that it more closely approaches the Wassermann reaction.

1. *Complement* is furnished by the fresh, clear serum of the guinea-pig, put up in 40 per cent. solution, prepared by diluting 1 part of serum with $1\frac{1}{2}$ parts of normal salt solution. Dose, 0.1 c.c. (5 drops from a capillary pipet). Whenever possible it is always best to use a mixture of the serums from two or more guinea-pigs.

2. *Human Corpuscles*.—These are washed three times with normal salt solution, and used in dose of 1 c.c. of a 1 per cent. suspension. To a graduated centrifuge tube containing 9 c.c. of sterile 2 per cent. sodium citrate in normal salt solution add 1 c.c. of blood and shake gently. This amount of blood is easily secured by pricking the finger with a lancet and collecting the blood in the centrifuge tube up to the mark 10. This is centrifuged thoroughly, and the supernatant fluid drawn off. More saline solution is then added to the corpuscles, stirred up, and the mixture centrifuged. The washing is repeated once more, and the supernatant fluid discarded. The corpuscles are then suspended in sufficient salt solution to make a total volume of 100 c.c.

3. *Hemolytic Amboceptor*.—Antihuman hemolysin is prepared by immunizing rabbits with human cells, as described on p. 401. It is a difficult matter to secure a potent amboceptor; human erythrocytes are more toxic than sheep's cells for rabbits, and most animals produce but small amounts of the amboceptor. Hemagglutinins are produced in large amounts, and when using a low titer hemolytic serum, the test corpuscles are quickly agglutinated in small dense masses that are broken up with difficulty and that interfere greatly with hemolysis. With serums having a titer of 1 : 100 or over the agglutinins are not so much in evidence; a satisfactory reaction is best observed, therefore, with a potent amboceptor (1 : 100) serum.

The hemolytic serum may be preserved in 1 c.c. ampules after adding an equal part of glycerin, and a stock dilution prepared and titrated in the usual manner. The serum is also well preserved dried on filter-paper, as

described on p. 57. A trial titration should always be made to determine the potency of the serum before the paper slips are prepared.

If paper amboceptor is used, the uniform rule of titrating it with the complement and corpuscles on hand should be observed before the actual tests are made. This is done chiefly because, where one guinea-pig serum is used for complement, it may occasionally happen that the serum is less active than usual, so that if fixed doses of complement and amboceptor are used, the reactions may at times prove to be incomplete and inaccurate. The process of titration is so simple that any one may readily conduct it, and thus fulfil the most important requirement of any complement-fixation test, namely, adjustment of the complement, amboceptor, and corpuscles to one another.

Titration of Serum Hemolysin.—Prepare a 1 : 100 dilution by mixing 0.1 c.c. of immune serum (inactivated) with 9.9 c.c. of saline solution. To a series of six small test-tubes add increasing amounts of this diluted serum as follows:

Tube 1: 0.1 c.c. amboceptor serum (1 : 100) + 0.1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 2: 0.2 c.c. amboceptor serum (1 : 100) + 0.1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 3: 0.4 c.c. amboceptor serum (1 : 100) + 0.1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 4: 0.5 c.c. amboceptor serum (1 : 100) + 1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 5: 0.8 c.c. amboceptor serum (1 : 100) + 0.1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 6: 1 c.c. amboceptor serum (1 : 100) + 0.1 c.c. complement (40 per cent.) + 1 c.c. corpuscle suspension (1 per cent.).

Sufficient saline solution is added to the first tubes of the series to bring the total volume up to 2 c.c. The tubes are then shaken gently and placed in the incubator at 37° C. for two hours (or one hour in water-bath at the same temperature), during which time they should be inspected and shaken gently several times. At the end of the period of incubation that tube which shows just complete hemolysis contains one amboceptor unit, and double this amount is used in making the main tests. If the serum has a titer of less than 1 : 500, it should not be used either in preparing the amboceptor slips or in conducting the reaction.

Titration of Dried Amboceptor Paper.—After the paper (S. & S. No. 597) has been evenly saturated with immune serum and dried, the sheets are cut into 5 mm. strips and standardized by placing increasing lengths of paper into a series of tubes as follows:

Tube 1: 1 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 2: 2 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 3: 3 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 4: 4 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 5: 5 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

Tube 6: 6 mm. paper + 0.1 c.c. complement (40 per cent.) (5 drops) + 1 c.c. corpuscle suspension (1 per cent.).

One cubic centimeter of saline solution is added to each tube, and the

mixture shaken gently and incubated at 37° C. for two hours or one hour in a water-bath. *At the end of this time the tube just completely hemolyzed contains one amboceptor unit, and in performing the test double this amount is used.* (See Fig. 142.)

This titration should always be conducted before the actual tests are set up, as is the rule in conducting the Wassermann reaction. When the titer of the paper is known, it may not be necessary to set up all the tubes of the foregoing series, as a few only are necessary to determine if the same amount of paper as was used in the previous tests will suffice with the new complement and corpuscle suspension at hand.

All titrations and the main tests may be conducted in a water-bath (37° C.). With the aid of a good thermometer a satisfactory bath is easily improvised. In fact, I believe that better results are secured in the water-bath than in the incubator. It is possible, therefore, to conduct these reactions in a perfectly satisfactory manner without the aid of an expensive incubator.

4. **Antigen.**—Acetone-insoluble lipoids (Noguchi) are to be used exclusively if the tests are conducted with active serums. When heated serums are used, any extract may be employed, as in making the Wassermann reaction, but the same antigen gives excellent results, and I use it exclusively in conducting the Noguchi reaction with both active and inactivated serums.

The antigen must be titrated as usual, and its anticomplementary, hemolytic and antigenic properties determined. According to Noguchi, an extract is satisfactory if it is antigenic in 0.02 c.c. of a 1 : 10 dilution, and not anticomplementary or hemolytic in amounts under 0.4 c.c. (1 : 10). In conducting the tests five times the antigenic unit, or 0.1 c.c., is employed; this dose is at least four times smaller than the anticomplementary unit, and is therefore safe and satisfactory.

The antigen is best preserved in methyl alcohol, as described on p. 457. Dried on paper and properly preserved in sealed tubes in a cold place it will retain its activity for several months, but as a general rule it is best to use fresh emulsions of the alcoholic solution.

Titration of Antigen.—The anticomplementary, hemolytic, and antigenic doses of an extract are determined in the same general manner as was described under the Wassermann reaction.

1. *Anticomplementary Titration.*—A portion of the stock alcoholic solution of acetone-insoluble lipoids is diluted with 9 parts of saline solution. This is the emulsion that is employed in conducting the Noguchi reaction, and contains 0.3 per cent. of the original lipoidal substances.

Sufficient emulsion for these titrations is prepared by diluting 0.4 c.c. of the alcoholic solution with 3.6 c.c. of saline solution.

Into a series of seven small test-tubes place increasing amounts of this emulsion as follows: 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 c.c., add 0.1 c.c. (5 capillary drops) complement (40 per cent.) to each; also 1 c.c. of a 1 per cent. suspension of corpuscles and sufficient saline solution to make the total volume in each tube about 2 c.c. Incubate at 37° C. for one hour (one-half hour in water-bath), and add two units of amboceptor. Shake the tubes gently and re-incubate for two hours (one hour in water-bath). That tube showing beginning inhibition of hemolysis contains the anticomplementary dose, which should not be under 0.4 c.c. In the tubes containing the larger doses slight hemolysis may be noticed, which is evidence of the hemolytic action of the extract.

An eighth tube should be included, containing 0.1 c.c. diluted complement, two units of amboceptor, and 1 c.c. of the corpuscle suspension. This is the hemolytic control and should show complete hemolysis.



FIG. 142.—TITRATION OF ANTIHUMAN HEMOLYTIC AMBOCEPTOR.
Shows complete hemolysis with 4 mm. of paper (unit).



FIG. 143.—NOGUCHI MODIFICATION OF THE WASSERMANN REACTION.
Positive reactions in tubes 1 and 3.

2. *Antigenic Titration.*—Since the extract is likely to have a high antigenic value, it is necessary to dilute the antigen still further by placing 0.5 c.c. of the foregoing emulsion in a test-tube and adding 4.5 c.c. of saline solution (1 : 100 dilution of the antigen). Into a series of six test-tubes place increasing amounts of this emulsion as follows: 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 c.c. To each tube add 4 drops (0.08 c.c.) of inactivated or 1 drop (0.02 c.c.) of fresh active syphilitic serum; also 0.1 c.c. (5 capillary drops) of complement (40 per cent.) and 1 c.c. of 1 per cent. corpuscle suspension. Then add sufficient salt solution to bring the total up to 2 c.c.

Two controls should be included: (1) The serum control, containing the dose of serum, 0.1 c.c. of the complement, 1 c.c. of corpuscle suspension, and saline solution; (2) the hemolytic control, containing at this time 0.1 c.c. of complement, 1 c.c. of corpuscle suspension, and sufficient saline solution.

All tubes are incubated for one hour at 37° C. (one-half hour in water-bath), after which two units of amboceptor are added to each tube. The tubes are then shaken gently and re-incubated for two hours (one hour in water-bath). At the end of this time the two controls should be completely hemolyzed, and in the series proper that tube showing just complete inhibition of hemolysis contains one antigenic unit. Usually the first and second tubes show some inhibition of hemolysis, and in the third and other tubes hemolysis is completely inhibited. In this case 0.2 c.c. of this emulsion would be one antigenic unit (= 0.02 c.c. undiluted antigen); five times this amount equals 0.1 c.c. of the first emulsion (1 : 10), which is the amount to be used in making the main tests.

Unless the antigen shows signs of deterioration, these titrations need be made only about once a month.

If paper antigen is employed, both titrations are conducted in exactly the same manner by adding increasing lengths of a strip of dried paper 5 mm. in width, impregnated with the antigen.

5. *Fluid to Be Tested.*—If active serum is used, it should be fresh, free from hemoglobin, and preferably not over twenty-four hours old. The dose is 0.02 c.c., or 1 capillary drop; inactivated serums are used in doses of 0.08 c.c., or 4 capillary drops. Cerebrospinal fluid is used unheated in doses of 0.2 c.c., or 10 capillary drops. Sufficient blood for this test may be collected in a Wright capsule. (See p. 17.)

6. *The Test.*—The complement, amboceptor, antigen, and serums may be conveniently measured by drops from a capillary pipet (Fig. 2). In placing a drop the pipet should be held uniformly at an angle of 45 degrees, or else the size of the drop will differ, depending on whether the pipet is held vertically or horizontally.

Arrange four pairs of small test-tubes (10 by 1 cm.) in a rack containing two rows of holes. Into each of the tubes on the front row place 5 drops (0.1 c.c.) of antigen emulsion (alcoholic solution, 1 part, with saline solution, 9 parts); then add 5 drops (0.1 c.c.) of complement (40 per cent.) to all the tubes. Into each of the first pair of tubes place 1 drop (0.02 c.c.) of active or 4 drops (0.08 c.c.) of inactivated patient's serum, and mark the front tube with the patient's name. To each of the second pair of tubes add an equal amount of syphilitic serum known to give a positive reaction (positive control), and to each of the third pair add normal serum known to give a negative reaction (negative control). Mark the tubes in the front row of each pair respectively. The front tube of the fourth pair is the antigen control, and the rear tube the hemolytic control, and each should be so labeled. Into each tube place 1 c.c. of the 1 per cent. corpuscle suspension and 1 c.c. of saline solution, making the total volume in each tube about

2 c.c. Shake each tube and incubate at 37° C. for one hour (half an hour in the water-bath). At the end of this time add two units of amboceptor to each tube, shake gently, and re-incubate for two hours (one hour in the water-bath). During this time the tubes should be shaken gently once or twice to break up any masses of agglutinated corpuscles.

The following chart, after Noguchi, illustrates the various steps to be taken in making the test with one patient's serum. Of course, any number of serums may be examined with the same controls (Fig. 143).

NOGUCHI MODIFICATION OF THE WASSERMANN REACTION

SET FOR DIAGNOSIS.	POSITIVE CONTROL SET.	NEGATIVE CONTROL SET.	ANTIGEN AND HEM- OLYTIC CONTROLS.	Incubate at 37° C. for one hour. (One-half hour in water-bath.)	Add 2 units of antihuman amboceptor to each tube.	Incubate two hours at 37° C. (One hour in water-bath.)
2.	4.	6.	8.			
Unknown serum: 1 drop. ¹ Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Positive serum: 1 drop. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Normal serum: 1 drop. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Hemolytic con- trol: Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).			
1.	3.	5.	7.			
Antigen: 5 drops. Unknown serum: 1 drop. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Antigen: 5 drops. Positive serum: 1 drop. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Antigen: 5 drops. Normal serum: 1 drop. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).	Antigen control: Antigen: 5 drops. Complement: 5 drops. 1 per cent. cor- puscle suspen- sion: 1 c.c. Salt solution (q. s. 2 c.c.).			

At the end of the second incubation, or after two hours more at room temperature, the tubes are inspected. The antigen and hemolytic system controls, as well as all the rear tubes or serum controls, should be completely hemolyzed. The first tube containing a known syphilitic serum shows inhibition of hemolysis; the front tube containing normal serum is completely hemolyzed; the front tube containing the patient's serum shows complete inhibition of hemolysis (strong positive), varying degrees of inhibition (moderately or weakly positive), or is completely hemolyzed (negative). The results may be recorded and reported after the same manner described on p. 472.

MODIFICATION OF CRAIG

Craig² employs a technic differing from the original Wassermann reaction in the use of a human hemolytic system in place of a sheep hemolytic system, and in a proportional reduction in the quantities of reagents used. Alcoholic extract of syphilitic liver is generally employed as antigen, although cholesterinized extracts of normal heart muscle have been found equally satisfactory.³ Vedder⁴ has also used Craig's method in the military ser-

¹ Four drops if serum is inactivated.

² War Department, Bulletin No. 3.

³ Amer. Jour. Med. Sci., 1915, cxlix, 41.

⁴ War Department, Bulletin No. 8.

vice, and it is widely used in the Army laboratories with satisfactory results.

Corpuscles.—A 5 per cent. suspension of washed human corpuscles preferably those belonging to Group IV (Moss classification) as recommended by Williams.¹ Dose, 0.1 c.c.

Hemolysin.—Rabbit antihuman serum dried in paper. In a series of six small test-tubes place increasing amounts of paper as follows, measured in millimeters: 5 x 1, 5 x 2, 5 x 3, 5 x 4, 5 x 5, and 5 x 6. To each tube add 0.1 c.c. of 1 : 5 complement, 0.1 c.c. of 5 per cent. corpuscle suspension and 0.9 c.c. salt solution. Incubate in a water-bath at 37° C. for one hour. A satisfactory paper should show complete hemolysis in a piece 5 x 5 mm. or less.

Complement.—This is titrated each time tests are conducted. Dilute guinea-pig serum complement with $1\frac{1}{2}$ parts of normal salt solution and place the following amounts in a series of nine test-tubes: 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.1 c.c. Add two units of hemolysin paper, 0.1 c.c. of 5 per cent. suspension of corpuscles, and 0.9 c.c. salt solution to each tube. Incubate in a water-bath at 37° C. for one hour.

Antigens.—Alcoholic extract of syphilitic liver and alcoholic extract of normal human heart reinforced with 0.4 per cent. cholesterin are recommended. Each antigen diluted 1 : 10.

Antigens are titrated for hemolytic activity in amounts of 0.05, 0.1, 0.15, and 0.2 c.c. of 1 : 10 dilutions in a series of test-tubes with two units of complement, 0.1 c.c. of 5 per cent. suspension of corpuscles and 0.9 c.c. salt solution; water-bath incubation for one hour. None of the tubes should show hemolysis.

The anticomplementary titrations are conducted by placing 0.05, 0.1, 0.15, and 0.2 c.c. of 1 : 10 dilutions in a series of test-tubes with two units of complement and 0.9 c.c. of salt solution; water-bath incubation for one hour. Two units of hemolysin and 0.1 c.c. of 5 per cent. corpuscle suspension are then added and the tubes re-incubated for one hour. There should be complete hemolysis in all tubes.

The antigenic titrations are conducted in the same manner except that 0.1 c.c. of inactivated luetic serum is placed in all tubes before the primary incubation. A satisfactory antigen is one giving complete inhibition of hemolysis in all tubes. A serum control should be included.

Hemolytic system and corpuscle controls should be included in all titrations.

If 0.05 c.c. of 1 : 10 antigen gives complete inhibition of hemolysis this amount is employed in the main tests; if 0.1 c.c. is the smallest amount giving complete inhibition this amount may be employed providing it is not anticomplementary in dose of 0.2 c.c.

Serum.—Heated at 55° C. for one-half hour; dose, 0.1 c.c.

Tests.—For each serum arrange three test-tubes. Place required amount of antigen of alcoholic syphilitic liver in the first tube, and the cholesterolized antigen in the second; the third tube is the serum control. Place 0.1 c.c. heated serum in each of the three tubes followed by two units of complement and 0.9 c.c. of salt solution. Water-bath incubation for one hour followed by the addition of two units of hemolysin and 0.1 c.c. of 5 per cent. corpuscle suspension. Re-incubate for one hour, followed by settling of the corpuscles in a refrigerator overnight.

Include controls with known positive and negative sera; also antigen, hemolytic system and corpuscle controls.

¹ Jour. Exper. Med., 1920, 32, 159.

Readings are made as follows:

++ = complete inhibition of hemolysis.

+ = anything between complete and 50 per cent. inhibition of hemolysis.

= = anything between 50 per cent. inhibition and complete hemolysis.

— = complete hemolysis.

MODIFICATION OF HECHT-WEINBERG-GRADWOHL

In conducting the syphilitic reaction Hecht¹ utilizes not only the natural antishoop amboceptor in human serum but also the native hemolytic complement. The serum must be perfectly fresh and, of course, is used unheated. This modification has been said to be more delicate than the Wassermann reaction because none of the syphilis antibody is destroyed or complementoids produced, as presumably will occur during inactivation (heating) of a serum. In my experience this test has proved quite delicate, but is open to the same error that may occur whenever an active serum is used with a crude alcoholic organic extract as antigen—i. e., the appearance of false positive or proteotropic reactions. As with the Noguchi reaction, using active serum, a negative Hecht-Weinberg test has considerable diagnostic value in excluding syphilis; a positive reaction must be, however, carefully controlled. In performing the test I always use an extract of acetone-insoluble lipoids as antigen.

Since in the original Hecht-Weinberg² test there is no way of determining beforehand the amount of sheep corpuscles a serum may hemolyze, Gradwohl³ has modified the technic so that the hemolytic index of each serum is determined before the corpuscles are added to the main tubes. I conduct this test as follows⁴: Nine small sterile test-tubes (10 x 1 cm.) are arranged for each serum and properly labeled; into each is placed 0.1 c.c. of fresh unheated serum. To the first five tubes are added respectively 0.1, 0.2, 0.3, 0.4, and 0.5 c.c. of a 5 per cent. suspension of washed sheep cells; to the sixth, seventh, and eighth tubes are added 1, 2, and 3 units of antigen respectively, as determined by titration. The last or ninth tube serves as the serum control. Sufficient normal salt solution is added to each tube to bring the total volume to 1 c.c.

After one hour's incubation at 37° C. the *hemolytic index* of each serum is read in the first five tubes of each series (that is, the largest dose of corpuscles just completely hemolyzed by each serum) and one-half the indicated doses of corpuscles added to the remaining four tubes of each series. After re-incubation for one-half to one hour, according to the hemolysis of the controls, the results are read and recorded as in the Wassermann reaction.

The *antigen titrations* are very important because the activity of human serum is quite sensitive to the inactivating influence of tissue extracts. No antigen should be employed in this test without preliminary titration with *human serum* to determine its anticomplementary and antigenic units. The *anticomplementary titration* is conducted by placing in a series of eight test-tubes the following amounts of antigen of acetone-insoluble lipoids diluted 1 : 100: 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5 c.c., and 0.1 c.c. of the fresh and mixed sera of two non-syphilitic persons; normal salt solution is added to 2 c.c. and the tubes incubated for an hour at 37° C., when one-half the index

¹ Wien. klin. Wchn., 1909, xxii, 265.

² Wien. klin. Wchn., 1908, 21, 1742; *ibid.*, 1907, 22, 265; *ibid.*, 1909, 22, 338.

³ Jour. Amer. Med. Assoc., 1914, 63, 240.

⁴ Jour. Immunol., 1916, 2, 23.

of cells for the serum is added to each tube. After a second incubation of an hour the anticomplementary unit is read as the smallest dose of antigen producing inhibition of hemolysis. The *antigenic titration* is conducted in the same manner with the serum of one or two syphilitic persons and the following doses of antigen (1 : 100): 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 c.c. The antigenic unit is the smallest amount giving complete inhibition of hemolysis.

THE CLINICAL SIGNIFICANCE OF THE WASSERMANN REACTION

The Necessity for a Proper Understanding of the Wassermann Reaction.—In their attitude toward the Wassermann test physicians may be divided into those who have considerable confidence in the reliability of the test and especially in the significance of a positive reaction both in the diagnosis of syphilis and as a guide to treatment, those who have little or no faith in the test in either a positive or negative way and those who have found it generally satisfactory providing weakly positive reactions are disregarded. Probably there is no other laboratory test the subject of as much disagreement between clinician and laboratorian; this is to be particularly regretted because no other strictly laboratory test possesses an equal importance or greater possibilities for good as a diagnostic means of a disease which is everywhere on earth among all classes of human beings, irrespective of sex and age. The disagreement and misunderstanding of the true value of the Wassermann reaction are due to biologic and technic sources of error, a correct understanding of which by both clinician and laboratorian, is essential for the proper practice and interpretation of this valuable test.

The Biologically Non-specific Nature of the Wassermann Reaction.—In the first place and as discussed in the preceding chapter on the nature of the antibody concerned, the complement-fixation test in syphilis as ordinarily practised is not biologically specific as are other complement-fixation tests. The reason is that the so-called "antigens" are not true antigens, that is, need not and usually are not, prepared of syphilitic tissues or the *Treponema pallida*. In all other complement-fixation tests the antigen employed must be an extract of the microparasite or other protein producing the disease.

Instead of using an extract or suspension of *Treponema pallida* for this test, the so-called "antigen" is nothing more than extract of tissue lipoids, and while these may be secured from either syphilitic or healthy tissues, the best "antigens" are probably prepared by extracting non-syphilitic tissues, as beef heart muscle.

The reaction in syphilis depends upon the peculiar nature of the so-called antibody in serum and spinal fluid which is endowed with the property of fixing complement in the test-tube in the presence of these lipoids.

Therefore, since the reaction in syphilis is not biologically specific because the antigen is not, the question arises: Does the reaction possess practical specificity for syphilis? The answer depends upon whether the peculiar lipotropic antibody-reagin is found in diseases other than syphilis.

THE OCCURRENCE OF THE WASSERMANN REACTION IN NON-SYPHILITIC CONDITIONS; THE PRACTICAL SPECIFICITY OF THE WASSERMANN REACTION

Unfortunately, the reaction is beset by so many technical errors that a review of the literature, and especially of the early literature, shows results that are quite confusing and contradictory. Following the original communications of Wassermann and Detre, and especially after it was demonstrated that the antigen need not be biologically specific, the subject was

extensively investigated by various observers, who reported securing positive reactions in many different diseases, results that we now know must have been due largely to technical errors. At present it is known that positive Wassermann reactions may occur in a few diseases other than syphilis, but not to the extent that earlier investigators would have us believe. In most of the diseases yielding positive reactions the clinical symptoms are so marked that they may readily be differentiated from syphilis, and accordingly the Wassermann reaction is of unequaled and incalculable diagnostic value.

Positive reactions have been reported in *frambesia* (yaws), in which the causal micro-organism, the *Spirochæta pertenuis*, is morphologically almost indistinguishable from *Spirochæta pallidum*. In *leprosy* of the tuberculous type positive reactions are frequently found, but in my experience these occur only occasionally and only in those lepers who are likewise syphilitic. Positive reactions have been reported in cases of *malaria* during the febrile stage, when parasites are present, although the majority of cases react negatively. In my own series of 11 cases all the reactions were negative. Positive reactions have also been found in some cases of *relapsing fever* and *trypanosomiasis*.

In *scarlet fever* the Wassermann reaction is uniformly negative. Owing to the original communication of Much and Eichelberg, however, in the minds of many this disease is prominently associated with a positive reaction. While it is true that a positive reaction is very rarely found, it is almost impossible entirely to exclude a diagnosis of congenital lues, at least in some of these cases. In my own series of 250 cases examined by the Wassermann and Noguchi methods, with antigens of alcoholic extract of syphilitic liver and acetone-insoluble lipoids, the reactions were positive in 5 cases, or 2 per cent. Similar results have been secured by Boas, Browning and Mackenzie, and others, so that it may be said that the reaction in scarlet fever is uniformly negative.

Normal cerebrospinal fluid or the fluid from persons with ordinary non-syphilitic diseases reacts negatively. Positive reactions occur in *frambesia*.

Positive reactions have also been reported in *tuberculosis of the lungs* and especially with cholesterolized antigens. This is certainly not my experience. Wassermann tests conducted with a large series of cases are almost sure to show a small percentage of positive reactions, but these occur among syphilitic individuals with tuberculosis.

Positive reactions have also been reported in *late pregnancy*, that is, the serum of a woman reacts positively during the late months of pregnancy and negatively after delivery. I believe this may occur occasionally with antigens containing 0.4 per cent. cholesterol, but not with plain antigens or those containing 0.1 or 0.2 per cent. cholesterol. In so far as my own experience is concerned, pregnancy of itself does not yield a falsely positive reaction at any stage. It is possible and, indeed, probable that pregnancy may stimulate latent foci of syphilitic infection into activity, resulting in the serum yielding positive Wassermann reactions, but these generally persist for several weeks after delivery before subsiding again into latency or for longer periods of time and are not to be interpreted as falsely positive. In my opinion a positive reaction in pregnancy when the test has been technically correct indicates the presence of syphilis.

Falsely positive reactions have also been reported as occurring in non-syphilitic persons with *diabetes mellitus*, *jaundice*, and *uremia*. In these conditions anticomplementary substances may be present in the serum

increasing the chances of securing falsely positive reactions, but in so far as my own experience is concerned the reactions are uniformly negative in the absence of syphilis.

Biologic and Technical Reasons for Positive Reactions in Non-syphilitic Diseases.—In frambesia tropica (yaws) the positive Wassermann reactions are apparently due to the same kind of lipotropic "reagin" as occurs in syphilis; this is to be expected because the *Treponema pertenue* causing this disease is biologically closely related to *Treponema pallida* and subject to the destructive influence of arsphenamin to even greater degree. It is possible that the positive reactions occurring in relapsing fever and trypanosomiasis are due to the presence of similar "reagins" and it is to be expected that positive reactions may occur in other spirochetic infections. In other words, there are biological reasons for positive Wassermann reactions in these non-syphilitic diseases, but *in localities where frambesia and relapsing fever do not occur at all or but seldom, the practical specificity of the Wassermann reaction for syphilis is well established.*

The complement-fixation test for syphilis is so complicated that there are numerous sources of technical error, but these can be reduced to a minimum and rendered negligible by experience and skill. Probably the use of cholesterolized "antigens" are most important in this connection. It cannot be denied that alcoholic extracts saturated with cholesterol and employed in relatively large amounts may yield falsely positive reactions with a small percentage of normal sera. But I am convinced that these results can be prevented by proper technical procedures. It is not necessary to use more than 0.2 per cent. cholesterol; the extracts should be frequently titrated and none should be used unless the dose of two or more antigenic units employed is at least one-fifth and preferably one-tenth of the anti-complementary unit.

Biologic and Technical Reasons for Falsely Negative Reactions in Syphilis.—It is readily understood that since the Wassermann reaction is due to the presence of "reagin" in the blood or spinal fluid, or both, that sufficient of this substance must be present before true positive reactions may be obtained. Therefore, in the primary stage of syphilis the reaction may be negative until the body cells are sufficiently stimulated to produce demonstrable amounts of this "reagin." As stated in the preceding chapter I believe this "reagin" is produced by the tissue-cells surrounding the spirochetes wherever they may be located and for this reason the "reagin" is to be found in the secretions of the chancre before it may be detected in the blood. For the same reason the "reagin" may be found in the spinal fluid and not in the blood of certain cases of syphilis.

Likewise in cases of latent syphilis either acquired or congenital, the spirochetes may be so quiescent that demonstrable amounts of "reagin" are not being produced. A certain amount of this substance must be present for positive reactions and in some cases of syphilis it would appear that the infection is so mild and latent that too small amounts are produced for detection in the complement-fixation test.

Of course falsely negative reactions may be due to faulty technic, that is, the technic may not be sufficiently sensitive. Every serologist endeavors to avoid falsely positive reactions and arranges his technic with this in mind; all will agree that it is better to miss the occasional case of syphilis than to secure a falsely positive reaction, but this may be carried too far. It is possible to render the complement-fixation test for syphilis very sensitive within the bounds of specificity and this should be the aim of all who conduct the test.

Negative Wassermann Reactions Do Not Exclude the Possibility of Syphilis.—Since there may be a biologic reason for negative Wassermann reactions in syphilis and numerous technical reasons, too much emphasis cannot be laid upon a negative reaction and especially a single negative result, in excluding the disease. *This is especially true during the primary and latent stages (both acquired and congenital infections) of the disease when "reagin" production has not occurred to sufficient degree to permit its detection in the blood and spinal fluid by complement-fixation tests.* The syphilis complement-fixation test as ordinarily practised is not too sensitive; rather it is not sensitive enough, always bearing in mind that falsely positive reactions due to faulty technic are to be avoided.

In my experience the reaction is but seldom negative in the presence of active syphilis after the primary stage. For this reason consistently negative reactions in the presence of an active pathologic process indicates the non-syphilitic nature of the latter with a great degree of accuracy providing always that the technic is sufficiently sensitive and correct.

Positive Spinal Fluid and Negative Blood Reactions.—It is now well established that in some cases of syphilis and especially those with involvement of the brain and spinal cord, the Wassermann test with the blood-serum may yield a negative reaction while the spinal fluid reacts positively. In long-standing and latent infections and especially in cases presenting clinical evidences of involvement of these tissues, a negative serum reaction possesses little or no value in excluding syphilitic infection. The spinal fluid requires examination and should be subjected not only to the complement-fixation test, but likewise to the colloidal gold test, protein determinations, and the counting of cells.

Furthermore, in many cases of treated syphilis the serum reaction may be negative when the spinal fluid reacts positively; for this reason an examination of the latter should be included before conclusion can be reached on the cure of syphilis in so far as this opinion is based upon serologic reactions.

The reasons for the positively reacting spinal fluid and negatively reacting serum are not understood. Since spinal fluid is used in the Wassermann test unheated and in amounts four to five times more than is permissible with serum, I believe that the chances for detecting the "reagin" in the spinal fluid are much greater and constitute the reasons explaining the reactions in some cases. Furthermore, it may be that "reagin" production occurs largely in the tissues of the brain and cord in syphilis of the central nervous system, and that it is thrown off into the spinal fluid rather than the blood, and that absorption of the "reagin" from the spinal fluid into the blood is reduced by pathologic processes.

A Positive Wassermann Reaction is Not Always an Indication that a Particular Lesion is Syphilitic.—Simply because an individual is known or suspected as being syphilitic does not exclude the possibility of the presence of other diseases. Not at all infrequently a non-syphilitic tumor or ulcer occurring in a syphilitic is regarded *a priori* as luetic; I have known this to occur in several cases of tuberculous ulcers of the larynx in syphilitic individuals. A positive Wassermann reaction, therefore, is an indication of syphilis, but does not necessarily mean that a particular lesion is syphilitic.

Syphilitic individuals are especially liable to attribute every ache and pain to syphilis; the "mental scars" of this disease are frequently incurable, but the physician must guard against the error of interpreting every disease process in a syphilitic as caused by *Treponema pallidum* and more especially guard against reaching such a conclusion on the basis of a positive

Wassermann reaction. Syphilis does not confer an immunity against other bacterial infections and pathologic processes; rather it may predispose to carcinoma, tuberculosis, and other diseases.

Biologic Reasons for the Unexpectedly Positive Wassermann Reaction; the Significance of Weakly Positive Reactions.—Not infrequently when the Wassermann test is conducted as a matter of routine the clinician is greatly surprised by a totally unexpected positive reaction. *This may be caused by technical error, but not infrequently is due to an error on the part of the physician, because syphilis may escape clinical detection, but yield a true positive Wassermann reaction.*

Cases of congenital and acquired syphilis insufficiently treated or not at all, but in the latent stages with indefinite lesions and symptoms or practically none at all, may occur in the practice of every physician irrespective of his special field of work and yield true positive Wassermann reactions. It is now so well known that syphilis may manifest itself in so many different ways that it may escape clinical detection. As Osler is so frequently quoted: "Know syphilis in all its manifestations and relations and all other things clinical will be added unto you." I am mentioning this phase of the subject in order to advise the physician against lightly brushing aside the unexpected or weakly positive reaction reported by a good laboratory, regardless of the sex or respectability of his patient; these results are not always due to technical errors in the laboratory and not infrequently indicate the presence of syphilis.

I believe that as *clinical and pathologic knowledge of syphilis is developed more and more emphasis will be placed upon the significance of weakly positive and unexpectedly positive reactions.* Furthermore, in the present state of our highly perfected technic every laboratory conducting the test should have enough faith in its results to place significance and confidence upon its weakly positive reactions.

Technical Sources of Error in the Wassermann Test and Variation in Results in Different Laboratories.—Confidence in the Wassermann reaction has been destroyed for many physicians by reason of securing varying reports from different laboratories with portions of the blood of the same person. This is very much to be regretted and constitutes an important reason for the adoption of a standardized test of superlative and proved merit.

These results, however, are not unexpected by serologists. The influence of technic upon the reactions is very great and especially in reference to the kind and amount of antigen employed, the hemolytic system and kind and duration of primary incubation, not to mention individual variations in skill and experience on the part of different laboratorians.

These discrepancies in reactions from different laboratories do not undermine the real value of the complement-fixation test in syphilis; they are largely the result of numerous modifications of technic being employed. To the average physician a Wassermann test is a Wassermann test irrespective by whom or by which method it is conducted; this is far from being true, and the physician should exercise care in the selection of the laboratory conducting these examinations.

THE WASSERMANN REACTION IN THE VARIOUS STAGES OF SYPHILIS

In general terms a sensitive complement-fixation test may be expected in my experience to yield approximately the following percentages of positive reactions with *serum* in the different stages of syphilis:

	Per cent.
Primary stage.....	92
Primary latent period (healing chancre; pre-eruptive stage).....	92+
Secondary stage.....	98-100
Secondary latent stage (after secondary lesions have subsided; usually some treatment had been received).....	86+
Tertiary stage with active lesions (exclusive of nervous system).....	96+
Tertiary stage with lesions of the central nervous system:	
(a) Paresis.....	96-100
(b) Tabes dorsalis.....	90+
Active prenatal (first year).....	100
Latent prenatal (after second year).....	80

1. **In Primary Syphilis.**—As would be expected, a certain degree of tissue change must occur before syphilis reagin appears in the blood. Even with the best technic there is a limit to the sensitiveness of the Wassermann reaction, so that while the reagin may be produced at the very onset of an infection, time and further tissue changes are required before sufficient reagin is produced to yield a complement-fixation reaction. As shown by Klauder and the writer,¹ however, tests conducted with chancre secretions are sometimes positive when the serum reactions are negative. It is possible that tests of this kind may possess diagnostic value, although there is usually some difficulty in obtaining sufficient fluid from the chancre for the test.

Since, therefore, the results of the Wassermann reaction in primary syphilis are dependent upon the virulence of the infection, the time at which the reaction is made, and the delicacy of the technic, it is not surprising that the results of different investigators vary in the proportion of positive reactions obtained. While positive reactions have been said to have been secured before the appearance of the initial lesion, these are rare, and there is always the likelihood that an earlier infection was overlooked. A careful review of our own work and the literature upon this subject establishes the following:

(a) A positive reaction may be secured during the first week after the appearance of the chancre. Craig has reported a positive reaction occurring five days after the appearance of the initial lesion. Levaditi, Laroche and Yamanouchi, and others have recorded many positive reactions occurring in ten days or more after the chancre made its appearance.

(b) In general, in primary syphilis the Wassermann reaction will be positive in about 80 to 92 per cent. of cases; where cholesterinized extracts are used as antigens, or with the Noguchi system, using active serum, the reactions are secured earlier and in a larger percentage of cases. Craig² has reported 34 per cent. positive reactions during the first week after the appearance of the chancre; 57 per cent. during the second week; 67 per cent. during the third week; 76 per cent. during the fourth week, and 80 per cent. during the fifth week.

(c) The cerebrospinal fluid of persons in the primary stage of syphilis has always reacted negatively (Boas).

Microscopic vs. Serum Tests.—It is generally agreed that a diagnosis should be made as early as possible, and vigorous treatment instituted. A Wassermann reaction may be performed, and if it shows a positive result, this indicates the presence of syphilis, even if the lesion under suspicion is not specific, the reaction being due to a previous infection. A negative reaction, however, does not exclude syphilis, and if it is at all possible, a

¹ Arch. Dermat. and Syph., 1922, 5, 566.

² Amer. Jour. Med. Sci., 1915, cxlix, 41.

microscopic examination, using the dark-ground illuminator, should be made for the treponema. In primary syphilis a microscopic examination of the secretions of the lesion by a competent person is usually more valuable than the serum test; as a general rule, both examinations should be made, especially with patients in whom the chancre is almost healed or atypical.

Klauder¹ has observed a series of cases especially valuable for bringing out the comparative values of microscopic and complement-fixation tests in primary syphilis; it is to be noted that the microscopic examinations were made with the dark-field illuminator and not by staining methods, which are of inferior value. His results with 115 cases were as follows:

Duration of chancre, days.	Per cent. positive dark field.	Per cent. positive Wassermann.
One to ten.....	93.9	36.0
Ten to twenty.....	52.9	64.7
Twenty to thirty.....	50.0	70.0
Thirty to forty.....	60.0	100.0
Over forty.....	30.0	100.0

It is worthy of note that the earlier the lesion, the more valuable the dark-field examination for diagnosis; as the lesion heals the Wassermann test increases in diagnostic value. The combined examinations will diagnose practically all cases. Repeatedly negative dark-field examinations with repeatedly negative Wassermann reactions exclude the syphilitic nature of a sore with great accuracy. Due care must be exercised in the microscopic examination of sores on the lips and in the mouth, as mouth spirochetes may be mistaken for *Treponema pallida*.

2. In **Secondary Syphilis**.—It is in untreated cases of secondary syphilis that the remarkable specificity of the Wassermann reaction is so well demonstrated. The initial lesion may have been inconspicuous and hence have been overlooked, and the secondary lesions may be quite mild and inconclusive; in either case the Wassermann reaction will usually establish the diagnosis.

(a) In untreated secondary syphilis the reaction is positive in from 98 to 100 per cent. of cases. In the examination of 437 serums from untreated cases Boas has never had a negative reaction, and my own experience has been the same. Craig reports 96 per cent. positive reactions.

(b) With the serums of patients who have received some treatment the percentage of positive reactions will be slightly lower. Of 310 such cases examined by Boas, 97.6 reacted positively. The influence of treatment upon the reaction is to be remembered, and a *single negative reaction does not by any means exclude the possibility of syphilis*.

(c) The intensity of the reaction does not bear any direct relation to the severity of the infection: a mild infection with indefinite signs may react quite strongly and absorb a large number of units of complement, whereas a severe case may react quite mildly.

(d) In secondary syphilis without cerebral symptoms the cerebrospinal fluid is practically always negative (Plaut, Boas, and Lind); conversely, cases showing cerebral involvement usually react positively. More recent work has shown that the cerebrospinal system is involved early and in a relatively large number of cases (Craig and Collins²). Udo J. Wile has found that about 30 per cent. of secondary syphilitics give a positive reaction with cerebrospinal fluid.

¹ Jour. Amer. Med. Assoc., 1919, lxxii, 694.

² Jour. Amer. Med. Assoc., 1914, lxii, 1955.

3. **In Tertiary Syphilis.**—It is probably in tertiary syphilis that the Wassermann reaction has its greatest value. Lues is so diverse in character, and may be responsible for so many diverse clinical conditions, that the reaction has become well-nigh indispensable as a diagnostic aid. There is no limit to the time following infection in which positive reactions may not be found.

(a) In cases of untreated and active tertiary syphilis the reaction is positive in about 96 per cent. of cases.

(b) In cases receiving more or less antisyphilitic treatment the reactions are positive in about 75 per cent. In general, therefore, a positive reaction in tertiary syphilis may be expected in from 80 to 95 per cent. of cases.

(c) In a large percentage of cases of syphilitic aortitis, aortic aneurysm, aortic insufficiency, gummas of various organs, etc., the reaction is positive and possesses great diagnostic value.

(d) The Wassermann reaction has been especially valuable in the study of the so-called *parasyphilitic diseases*. In *general paralysis* or paresis the serum reacts positively in about 100 per cent. of cases, and the cerebrospinal fluid reacts positively in from 96 to 100 per cent. The final and conclusive proof of the syphilitic nature of this disease has been furnished by Noguchi and Moore, who found the *Treponema pallidum* in sections of the brain. In certain cases of general paralysis the blood-serum may react negatively, whereas with the cerebrospinal fluid the reaction is positive.

The fact that the blood-serum of a patient with a nervous disease reacts positively does not necessarily indicate that the nervous disease is of syphilitic origin, as the reaction may be due to specific infection of some other structure; if, however, the cerebrospinal fluid also reacts positively, then it is almost certain that syphilitic infection of the central nervous system is present.

In untreated and active cases of *tabes dorsalis* the blood-serum reacts positively in from 96 to 100 per cent. of cases. In treated cases the number of positive reactions drops to about 50 to 80 per cent.; in general, a positive reaction with the serums of tabetics may be expected in 90 per cent. of cases. With the cerebrospinal fluid the percentage of positive reactions is somewhat lower, being about 85 per cent. The positive Wassermann reaction is less constant in locomotor ataxia than in general paralysis, due probably to the fact that the former is more chronic and that intercurrent periods of arrest are more prone to occur.

In *cerebral syphilis* the blood-serum, and particularly the cerebrospinal fluid, will react positively less frequently than in general paralysis. In some instances a positive reaction is found with the cerebrospinal fluid and not with the serum, a matter difficult to explain and believed to be due to the confining of the reacting substances in the subarachnoid space. On the other hand, the lesions are probably not brought in direct contact with the spinal fluid.

There is much evidence to indicate that localization of syphilis in the nervous system is dependent upon a particular strain of *Treponema pallidum*; other strains appear to possess a special affinity for the visceral organs, bones, etc.

(e) In tertiary syphilis not accompanied by lesions of the central nervous system the Wassermann reaction with cerebrospinal fluid may be positive in a relatively large percentage of cases.

4. **In Latent Syphilis.**—In cases of latent syphilis the Wassermann reaction may constitute the only evidence of the existence of the disease, and prompt institution of treatment may prevent the development of tertiary

lesions, which are so likely to follow. When the spirochetes are few in number and are dormant, there is little tissue destruction or alteration, and, as a result, so little reagin is frequently present in the body fluids that the Wassermann reaction will fail to detect the disease.

(a) In 363 cases of early latent syphilis, or those included within a period of three years after infection, Boas found positive reactions in about 40 per cent.; in latent cases of long standing, or in those following manifest tertiary lesions, the same investigator found 22 per cent. of positive reactions among those who had received proper treatment; of those receiving indifferent treatment, 74 per cent. reacted positively, giving a general average of about 48 per cent. Craig has found 67 per cent. positive reactions in latent syphilis; Vedder, 80.7 per cent.

(b) The reaction with cerebrospinal fluid depends upon whether or not the central nervous system is involved in the syphilitic process. Of 104 latent cases of syphilis in whom the spinal fluid was examined by Altman and Dreyfus, positive reactions were found in about 10 per cent.

5. Prenatal or Congenital Syphilis.—The Wassermann reaction has thrown considerable light upon the subject of congenital syphilis. While, in general, the majority of cases react positively, the results are largely dependent upon the time when the examinations are made, a fact brought out by highly instructive and systematic investigations of Boas and Thomsen. These investigators divided their cases into three groups: (1) New-born children and their mothers; (2) two-year-old children; (3) older children with congenital syphilis.

(a) Of 88 children born of syphilitic mothers and examined at birth, the reaction was positive in 31 and negative in 57 cases. Of the 31 positive cases, 4 showed no symptoms of syphilis for a period of observation covering from three to nine months, and it is possible that the syphilis reagin, and not the spirochetes, from the blood of the mother, passed into the circulation of the child; on the other hand, all 4 cases may have been examples of retarded congenital syphilis. The remaining 27 cases either developed symptoms of syphilis or died later with syphilitic manifestations in various organs.

Of the 57 children reacting negatively at birth, 42 showed no symptoms of syphilis during a period of three months of observation; 2 died with evidences of syphilis in the internal organs; 13 developed symptoms after birth and gave positive reactions.

It may therefore be stated that a Wassermann reaction of the mother and of the child at the time of birth in cases where syphilis of the mother is suspected has considerable prognostic value. A large majority of children reacting positively develop symptoms of syphilis; on the other hand, the majority reacting negatively remain healthy. While an examination of the mother alone does not warrant an absolutely definite prognosis for the child, in general it may be said that a positive reaction does not constitute a favorable prognostic sign for the child.

(b) The Wassermann reaction has also shed new light upon the interpretation of *Colles' law*. Since the "apparently healthy mother of a syphilitic child could suckle the child without being infected, whereas the child is capable of giving syphilis to others," the most logical conclusion to draw is that the mother was gradually immunized against syphilis during pregnancy, whereas we now know that the majority of mothers show positive serum reactions and are really latent syphilitics; in not a few such instances tertiary lesions have developed at a later date.

It is possible, however, for a syphilitic mother showing a positive Wasser-

mann reaction to give birth to a healthy child. Of 46 mothers whose children showed no evidences of syphilis over a period of observation of three months, 17 reacted positively. Of 81 mothers giving birth to syphilitic children, 61 reacted positively, and many of these would naturally, in former years, have been regarded as examples of Colles' immunity and considered free of syphilis. In many instances the apparently healthy child of a syphilitic mother that could not be infected by the mother (*Profeta's law*) has been shown by the Wassermann reaction to be in reality a case of retarded congenital syphilis, and that such children are not immunized, during intra-uterine life, either passively or by means of pallidum toxins, against syphilis, as has been so generally believed in past years. In other words, there appears to be no lasting passive immunity in syphilis; it is doubtful if the toxins of pallidum can pass between mother and child and immunize one or the other without actual infection with the spirochetes themselves taking place; that most examples of so-called immunity in syphilis in both the mother (Colles' law) and the child (Profeta's law) are due to the actual presence of pallidum in the tissues and are really latent infections.

(c) In manifest *untreated congenital syphilis* of children one year or over in age the Wassermann reaction is positive in from 97 to 100 per cent. of cases. The clinical manifestations may be quite varied and clinically ill defined, so that the serum reaction possesses considerable diagnostic value. In most instances the reactions are quite strong, and while active treatment may improve local lesions, it is very difficult, indeed, to secure negative reactions.

(d) In *congenital mental deficiency* and *epilepsy* the Wassermann reaction shows that syphilis plays a larger part in the etiology of this condition than is generally supposed. A not inconsiderable proportion of cases are of infectious origin, and that infection is syphilis. In Little's disease, which is regarded as due to meningeal hemorrhage incidental to injury received during labor, the serum reactions have shown that not infrequently the hemorrhage has a syphilitic origin.

THE EFFECT OF TREATMENT UPON THE WASSERMANN REACTION

Citron originally observed that during the mercurial treatment of syphilis the Wassermann reaction gradually became weaker, and finally disappeared. He also found that treatment was best governed by the serum reaction, and that it should be persisted in until a negative reaction was secured. His observations have in the main been abundantly confirmed by various observers the world over, although the extensive series of observations now on record have given us a fuller understanding of its principles.

The Wassermann reaction is the most constant and delicate single symptom of syphilis, usually the last to disappear under treatment and the first to reappear if complete sterilization has not been accomplished. It is now quite generally believed that a persistently positive reaction indicates the presence of living spirochetes, and that treatment should be continued until the blood reacts negatively. The reports of observers from all parts of the world indicate quite clearly and conclusively that the schematic, symptomatic, intermittent, and hard-and-fast rules of treatment of former days are not sufficient. They would also tend to show that the Wassermann reaction is the most delicate symptom and the last to disappear, and that treatment should be continued until this reaction disappears entirely and permanently. *It has been abundantly proved, however, that in syphilis a single negative reac-*

tion is not sufficient or definite evidence that a cure has been effected, for the disease may recur after treatment is discontinued, at least to the extent that the Wassermann reaction reappears, followed by clinical manifestations. It is necessary, therefore, that successive examinations be made during a period of at least two years, and off and on during the remainder of life. Recent work indicates that certain strains of *Spirochæta pallida* have an apparent selective affinity for the tissues of the central nervous system; the Wassermann reaction with blood-serum may be negative, whereas with the cerebrospinal fluid it may be positive. In cases, therefore, of tertiary syphilis, at least, it is advisable to examine the spinal fluid and continue treatment in case it shows a positive Wassermann reaction.

It should be the object of treatment, in every case, not only to dissipate the external and obvious lesions of the disease, but to produce a condition of the blood in which the Wassermann reaction is permanently negative. It is quite generally agreed that the older methods of treatment, consisting of the administration of mercury and the iodids over fixed and arbitrary periods of time, or until all manifest symptoms have disappeared, are insufficient, and that the criteria by which the effects of treatment can best be judged are: (1) Continued absence of symptoms, and (2) permanent negative Wassermann reactions.

It is to be remembered, therefore, that while a single negative reaction is a satisfactory indication of the progress of treatment, it does not signify that a permanent cure has been effected. The Wassermann reaction cannot be regarded as sufficiently delicate to indicate that a single negative reaction means that a patient is totally free from all spirochetes, for in some instances the reaction and the clinical symptoms may recur after the treatment has been suspended, but the reaction is the first symptom to reappear and the earliest indication of an impending lesion. For all practical purposes the occurrence of a negative reaction after treatment indicates either complete destruction of all the spirochetes, or at least that the parasites are being held in abeyance and rendered potentially harmless.

It is, accordingly, reasonable to regard the Wassermann reaction as the most delicate indicator of generalized spirochetal infection or the assumption of spirochetal activity. A positive reaction indicates that serious effects and gross local lesions are likely to occur at any time, and that treatment should be continued. For all practical purposes a continued absence of symptoms and a permanently negative reaction are strong presumptive evidences that a cure has been effected.

The serum should be tested every four months during the treatment, and at periods of at least six months to a year after treatment has been discontinued for several years. Persistently positive reactions during treatment would indicate that more active measures or a change in therapy are needed. The occurrence of a positive reaction after treatment has been discontinued is an indication for its resumption.

For a control on treatment the Wassermann reaction should be made as delicate as possible, for while more prolonged treatment may be somewhat irksome to the patient, it is clearly indicated as a preventive of serious after-effects, especially of involvement of the central nervous system. It is in this branch of the work I have found that the use of sensitive cholesterinized extracts as antigens in making the Wassermann reaction, of great value as the most delicate indicators.

One fact is to be clearly emphasized, namely, that the earlier energetic treatment is begun, the more likely it is that a permanent cure will be effected. Energetic treatment with mercurials or salvarsan, or, better, with a combination

of both, begun early and continued long, will in the majority of cases restore the serum to its normal condition. In general, the greater the interval of time allowed to elapse between infection and institution of treatment, the more difficult it is to restore the serum to normal. Tertiary cases are cured only as the result of most persistent treatment, and not infrequently in congenital syphilis, locomotor ataxia, and general paralysis all one can hope to accomplish is to check the progress of the disease. *The most favorable cases are those in which early diagnosis is made possible by clinical manifestations, preferably confirmed by a demonstration of pallidum, and in which treatment is undertaken before the serum has begun to react positively, and in which the reaction remains negative throughout.*

Treatment will, however, at least influence the Wassermann reaction in practically all stages of syphilis. In a series of 435 cases of syphilis in all stages reported by Boas, a negative Wassermann reaction was secured in no less than 80 per cent., and all but one of the remaining cases showed a weaker reaction. The figures of different observers are not all so favorable as these, a factor dependent to some extent, at least, upon differences in the technic of the reaction. In general, however, Boas' observations have been confirmed by other competent workers.

The effect of any treatment is greatly influenced by the individuality of the host, certain persons possessing tissues more amenable to the effects of the therapeutic agent than those of others. The therapeutic effect is also dependent upon the virulence of the parasite and the apparent selective affinity of certain strains of pallidum for particular organs, and upon the method of treatment selected.

The influence of salvarsan and neosalvarsan as agents in the treatment of syphilis is considered elsewhere. My experience has shown that the earlier belief in the complete sterilization of the human patient by a single dose was generally unfounded, and that repeated smaller doses of the drug, used in conjunction with mercurials, are necessary. Potassium iodid alone may favorably influence the clinical symptoms and weaken the Wassermann reaction in a small percentage of cases, and the same result has been observed with such arsenical preparations as Fowler's solution, atoxyl, arsacetin, and arsenophenyglycin.

It is to be remembered that, during or immediately after active treatment with salvarsan or mercury, the Wassermann reaction may be negative, even though the patient is not cured. As a general rule, a negative reaction under these conditions should not be considered of value unless all treatment has been omitted for at least two weeks; even then the test, if negative, should be repeated a month or so later. Craig has recently drawn attention to the fact that in frank untreated cases the degree of the reaction may vary within wide limits, and this is especially true if the patients are receiving active treatment.

Provocatory Stimulation.—Paradoxical as it would at first appear, anti-syphilitic treatment may convert a negatively reacting serum into a positive one. In not a few cases of latent syphilis reacting negatively the administration of a specific spirillicidal agent, such as mercury or salvarsan, is followed by positive reactions, due probably to the liberation of endotoxins from destroyed spirochetes or to a stimulation of the spirochetes by a dose of drug that did not suffice to kill them. This condition is analogous to the Herxheimer reaction, or the aggravation of skin lesions sometimes observed to follow the administration of mercury or salvarsan. The fact possesses practical value, for in cases where lues is known to have been present or is strongly suspected, and the Wassermann reaction is indefinite or negative,

the administration of 0.3 to 0.4 gm. of arsphenamin or neo-arsphenamin, followed by a Wassermann reaction twenty-four, forty-eight, and seventy-two hours later, may now show a positive reaction and thus indicate a latent syphilis requiring further treatment. Pollitzer and Spiegel¹ have not found this method of any value and believe that it may be misleading. Stokes and O'Leary² believe that it possesses value in certain cases.

PRACTICAL VALUE OF THE WASSERMANN REACTION

As previously stated, the Wassermann reaction serves two important purposes: (1) As an invaluable aid in the diagnosis and (2) as a guide in the treatment of syphilis.

The reaction may be of great value in determining the diagnosis of extra-genital sores and of atypical lesions in all stages of syphilis. A negative reaction, however, has less value than a positive one, and whenever possible, a microscopic examination of the secretions with the dark-field illuminator should be made in order to confirm the diagnosis. In early latent syphilis, after the initial lesion has healed, and before the secondary eruption appears, the Wassermann reaction is frequently the only means of making the diagnosis, especially if the chancre has been small, atypical, and practically neglected.

Indefinite symptoms and clinical unrecognizable cases constitute a considerable proportion of cases of syphilis, and, as is true in all other infections, this class constitutes the greatest menace to public health. Many patients are sincere in denying knowledge of infection and early symptoms may be overlooked, the Wassermann reaction being the sole means of diagnosis and serving in this connection as an invaluable aid.

Usually the symptoms of syphilis are so well marked in the secondary stage that the reaction is in most instances but confirmatory evidence. However, in doubtful cases a negative reaction excludes syphilis with almost absolute certainty, especially if the reaction is repeatedly negative.

In the late latent and tertiary stages of syphilis the Wassermann reaction may be the only available basis on which to establish a diagnosis. When one remembers how varied are the clinical manifestations of chronic syphilis, how wide-spread is the disease, and how frequently the reaction establishes the true diagnosis, the reaction must be regarded as being of great value and as an indispensable diagnostic aid. It must not be forgotten that patients showing an early involvement of the central nervous system, and even those showing no such symptoms, may react negatively with blood-serum and positively with spinal fluid; *in all such cases the spinal fluid should be examined whenever possible.*

A positive reaction occurring in aborting women is an indication for treatment and may protect the fetus. Similarly a positive reaction in either parent of a seemingly healthy infant is an indication for treatment of the child, especially if the mother reacts positively.

In this connection, however, one point is worthy of special emphasis, namely, that although a positive reaction indicates that the patient is luetic, it does not necessarily mean that a particular lesion is syphilitic. For example, a person may be luetic and yet have a cancerous ulceration of the larynx. The mere fact that the lesion does not improve under anti-syphilitic treatment does not detract from the value of the Wassermann reaction, but is a warning that more care is required in making the clinical

¹ Amer. Jour. Syph., 1919, 3, 252.

² Archiv. Dermat. and Syph., 1920, 2, 348.

examination. I have seen a number of such cases in which a positive Wassermann reaction was held *a priori* as evidence of the syphilitic nature of a lesion that later proved to be either malignant or tuberculous. A weak positive reaction, associated with an active ulcerating lesion, very frequently indicates that the lesion is not syphilitic, for active lesions usually yield strongly positive reactions.

In this connection may also be mentioned the growing importance the Wassermann reaction has assumed in life-insurance examinations. Statistics show that from one-tenth to one-third of all persons infected with syphilis die as the results of the disease, and the death-rate among 5000 syphilitics accepted for insurance was one-third over expectation (Brockbank).

An important question, especially from the standpoint of therapeutics, is: Does a positive reaction invariably indicate the presence of living spirochetes? May the reaction remain positive for an indefinite time after the patient has been cured, just as agglutinins and antitoxins may persist in the blood for some time after recovery from typhoid fever and diphtheria has taken place? The sum total of the experience of investigators from all parts of the world would indicate that a persistently positive reaction means the presence of living spirochetes somewhere in the body. The lesions may not be active; the patient, while clinically healthy, may be infective, and is always subject to possible recurrences of clinical syphilis.

Although gummas are slightly infectious, it is now known that they contain living spirochetes, and the former view, which regarded them as sequels, rather than as actual active lesions of syphilis, is no longer tenable.

Just how long the reaction may remain positive after the patient is actually cured and all spirochetes are dead is, of course, difficult to state, but experimental studies on the lower animals has shown that the reagin disappears somewhat quickly under these conditions.

Although a persistently negative reaction is of good prognostic importance, it is not so conclusive in the information it yields as is a positive reaction. In other words, an occasional active lues may react negatively, and not infrequently active syphilitic lesions are found at autopsy in persons whose blood reacted negatively during life. While it is true that great harm may result from a false positive diagnosis due to faulty technic, yet it must be admitted that the Wassermann reaction is not too delicate, and that we are just as prone to err on the side of securing too many negative reactions. Every effort should be made to render the test as delicate as is possible with specificity.

While the value and dependability of the Wassermann reaction are based upon skilful technic that will eventually limit the performance of the test to specially trained persons in central laboratories, every effort should be made to render accessible to all persons this valuable diagnostic test of a disease that has such great social and economic importance. At present many persons are unable to afford the expense of a number of tests, or even of one test, as required in the modern treatment of this disease. This deficiency should be corrected, and the test made available in all free dispensaries, especially those under the supervision of a Social Service Department.

CHAPTER XXIV

PRECIPITATION REACTIONS IN SYPHILIS

OWING to the complexity of the complement-fixation reaction for syphilis, the several sources of error, and the time required for conducting the test it is not surprising that early and numerous attempts have been made to simplify the serum diagnosis of this disease. The investigations of Morreschi, having shown that the phenomenon of complement fixation may be associated with precipitation, it was to be expected that similar flocculation tests should be applied in the serum diagnosis of syphilis, and especially since a considerable amount of evidence has accumulated in support of the hypothesis that complement fixation in syphilis is a phenomenon of flocculation of lipoids in colloidal suspension with fixation or absorption of complement. Michaelis¹ was among the first to note precipitation when heated syphilitic serum was added to diluted alcoholic extract of syphilitic liver; he erroneously regarded the phenomenon as a specific precipitin reaction. Since then a large number of similar tests have been described and compared with the complement-fixation test as diagnostic reactions for syphilis.

Classification.—These tests may be classified as follows:

1. *Specific precipitin reactions*, as the reaction of Fornet and Scherschewsky,² which was briefly described in Chapter XVII. This reaction was based upon the precipitation of pallida proteins in syphilitic sera by antisiphilitic sera secured from long-standing cases of syphilis, as individuals with paresis and tabes. The reaction is of interest from the standpoint of production of specific precipitins for pallida proteins in syphilis, but occurs infrequently and possesses no diagnostic value.

2. *Coagulating reactions* by chemical agents, as the butyric acid test of Noguchi, the nitric acid test of Bruck, the "gel" test of McDonagh, and the formol test of Gate and Papacostas.

3. *Colloidal flocculation reactions*, as the distilled water reaction of Klausner and the reactions of Hirschfeld and Klinger, Porges and Meier, Herman and Perutz, Meinicke, Vernes, Sachs and Georgi, Dreyer and Ward, Kahn, and others.

CHEMICAL COAGULATING REACTIONS

These reactions are mainly based upon the detection of protein changes in the serum and spinal fluid in syphilis. As shown by Rowe,³ and confirmed by Tokuda⁴ in my laboratory, refractometric studies have indicated that in syphilis there is an increase of the serum globulins. These changes, however, are not characteristic of syphilis, but have been found in other infectious diseases. In the spinal fluid an increase of protein, and especially of globulins, is known to occur in acute and chronic meningitis of bacterial origin as well as in some forms of neurosyphilis. The following tests applied to the diagnosis of syphilis have not proved specific for this disease because they are unable to measure quantitative changes; that of Noguchi, however, has proved a valuable means for the detection of increased protein of syphilitic or bacterial origin in the spinal fluid.

¹ Berl. klin. Wchn., 1907, xliv, 1477.

² Berl. klin. Wchn., 1908, 18, 874.

³ Arch. Int. Med., 1917, 19, 354.

⁴ Arch. Dermat. and Syph., 1921, 4, 512.

Noguchi's Butyric Acid Reaction.—Noguchi's test¹ depends upon the coagulation of proteins by butyric acid and is employed for the detection of an increase of these in cerebrospinal fluid. It was first employed for the detection of an increase of proteins in the spinal fluid in paresis, but has since proved to be generally useful for the examination of spinal fluids in other inflammatory conditions of the meninges.

In my experience this test has proved of particular value in establishing the differential diagnosis between serous and tuberculous meningitis, being negative in the former and positive in the latter, whereas in both the fluid may be clear, the cytology may be indefinite, and tubercle bacilli may escape detection. Serous meningitis is not a true infection, but a reflex vasomotor disturbance of the cerebral vessels, causing an outpouring of serum that leads to various pressure symptoms closely resembling those of a true meningitis. This condition is particularly common during child-

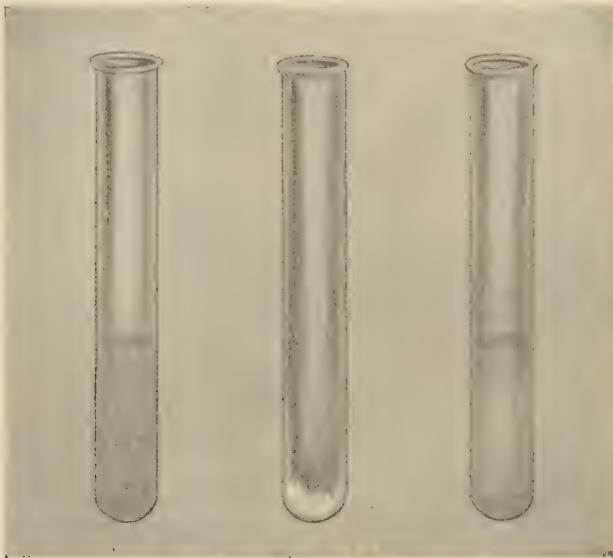


FIG. 144.—THE NOGUCHI BUTYRIC ACID TEST FOR GLOBULINS.

The tube on the extreme left shows the formation of floculi within a few minutes after adding NaOH; the middle tube shows a strongly positive reaction after standing several hours (supernatant fluid quite clear); the tube on the extreme right shows a very slight opalescence, but no floculi (within the limits of normal).

hood, and the general symptoms, the increased pressure of the cerebrospinal fluid, and its clear, watery character, are features that resemble those of tuberculous meningitis. It is just in such cases—and they are frequent—that I have found this protein reaction of considerable value. A positive reaction practically always means a true meningitis; a negative reaction usually means "serous meningitis," with a much better prognosis if the underlying cause is corrected.

Noguchi has found the test positive in about 90 per cent. of cases of general paralysis and in 60 per cent. of cases of locomotor ataxia or cerebral or spinal syphilis. In the diagnosis of syphilis the Wassermann reaction with cerebrospinal fluid has greater value than the protein reaction.

¹ Jour. Exper. Med., 1909, 11, 92.

However, the best results in diagnosis are usually secured by a Wassermann test, butyric acid test, and total and differential cell counts. In a case where the diagnosis rests between tuberculous meningitis and syphilis, a positive butyric acid test and a negative Wassermann reaction would decide in favor of the former.

The test is extremely simple. Into a small, thin-walled test-tube place 0.2 c.c. of cerebrospinal fluid (which must be clear and free from blood); add 1 c.c. of a 10 per cent. solution of butyric acid in normal salt solution; heat over a low flame and boil for a short period. Then add quickly 0.2 c.c. of a normal solution of sodium hydroxid and boil once more for a few seconds. The presence of an increased content of protein is indicated by the appearance of a granular or flocculent precipitate, which gradually settles to the bottom of the tube, under a clear supernatant fluid (Fig. 144).

The velocity and intensity of the reaction vary with the quantity of the protein contained in a given specimen. The granular precipitate appears within a few minutes in a specimen containing a considerable increase in protein, whereas one hour may be required to obtain a distinct reaction in specimens weaker in protein. In obtaining the reaction the time period should not be greater than two hours. *A faint opalescence without the formation of a distinct precipitate is to be regarded as within the limits of the normal.*

Bruck's Nitric Acid Reaction.—Bruck¹ has described a simple test for syphilis based on the observation that the precipitate formed from 0.5 c.c. of serum after the addition of 0.3 c.c. of a 25 per cent. dilution of nitric acid (specific gravity of 1.149) is not dissolved when 16 c.c. of distilled water are added ten minutes later, the tube inverted three times and stood aside for half an hour. This reaction has been investigated by Smith and Solomon,² Stillians,³ Terada,⁴ and others. Toyama and myself⁵ found that there was some difficulty in reading borderline reactions, that the test agreed with a sensitive Wassermann test in only 70 per cent. of cases, and that it yielded about 8 per cent. falsely positive reactions. Our conclusion was that this reaction was not as reliable as the Wassermann reaction.

McDonagh "Gel" Reaction.—McDonagh⁶ has advocated what he has designated a "gel" test for the diagnosis of syphilis. Clear and blood-free sera are employed, and it is necessary to include a known positive and negative serum each time the tests are conducted. Into each of three dry tubes place 2, 3, and 4 drops of serum, respectively; add 0.1 c.c. of acetic anhydrid and 1 c.c. of glacial acetic acid; the tubes are now well shaken and 1 drop of a saturated watery solution of ammonium sulphate added. Instead of acetic anhydrid and solution of ammonium sulphate the test may be conducted with 0.2 c.c. of a saturated solution of lanthanum sulphate, thorium sulphate, or thorium nitrate in glacial acetic acid. A preliminary reading may be made and a final reading after the tubes have stood over night. "In all the tubes at first crystals form, but in the tubes containing normal serum they often disappear in six to twenty-four hours, while they remain in the tubes with syphilitic serum." Strickler has used this test in my laboratory, and my observations of his results leads me to the conclusion that it is frequently difficult to interpret the reactions,

¹ Münch. med. Wchn., 1917, 64, 25.

² Boston Med. and Surg. Jour., 1917, 177, 321.

³ Jour. Amer. Med. Assoc., 1917, 69, 2014.

⁴ Kitasato, Archiv. Exper. Med., 1919, 3, 123.

⁵ Jour. Cutan. Dis., 1918, 36, 429.

⁶ Brit. Jour. of Dermat., 1916, April-June, 114.

and that the test has not by any means the practical value of the Wassermann reaction.

Formol Reactions.—Gate and Papacostas¹ have recently described a test for syphilis consisting of the addition of 2 drops of commercial formalin to 1 c.c. of clear serum. The mixture is allowed to stand for twenty-four to thirty hours at room temperature; coagulation is supposed to occur with syphilitic sera, but not with the sera of non-syphilitic individuals. Ecker² found that the test was of no value because of its failure to react in syphilis and the occurrence of positive reactions with the sera of non-syphilitic individuals. Burke,³ in a study employing both sera and spinal fluids, found the test unreliable and especially from the standpoint of yielding too many negative reactions. Spinal fluids from cases of syphilis were found regularly to yield negative reactions.

Suffern⁴ has described a similar test which in my laboratory yielded results quite similar to those reported by Ecker.

COLLOIDAL PRECIPITATION REACTIONS

Since the discovery by Michaelis that in mixtures of syphilitic serum and alcoholic extracts of tissues colloidal precipitation may occur, a large number of tests for syphilis have been devised on this principle. It is now generally accepted that suitable extracts for the Wassermann test depend upon the size of the colloidal molecules and their capacity or incapacity for flocculation. It is highly probable that in mixtures of serum, complement, and antigen that colloidal flocculation occurs which entangles or removes the hemolytic activity of the complement and thereby reduces the degree of hemolysis following upon the addition of hemolysin and corpuscles.

This colloidal flocculation by syphilitic serum (and by normal serum as well under certain quantitative conditions) in mixtures with alcoholic tissue extracts or synthetic extracts of bile salts or other lipoidal substances, can be detected either by (a) direct inspection macroscopically or microscopically or (b) indirectly measured by the degree of inhibition of hemolysis. In the latter instance a hemolytic serum must be added to the mixtures of syphilitic serum and antigen in order that the degree of hemolytic activity shall be reduced in proportion to the degree of colloidal flocculation; this principle is the basis of Vernes' indirect method.

Klausner's Water Reaction.—Klausner⁵ has described a test consisting of the addition of 0.6 c.c. distilled water to 0.2 c.c. of fresh, clear, and unheated serum. In seven to fifteen hours a flocculent precipitate forms which is more marked in syphilitic than in non-syphilitic sera. While the reaction was originally regarded as due to an increase of serum globulins, Klausner⁶ now believes that it is caused by lipoids in syphilitic sera and that heating an extraction with ether removes the reacting substances. The reaction, however, has not proved of practical value in the serum diagnosis of syphilis.

Hirschfeld and Klinger's Coagulo Reaction.—This test⁷ is based upon the hypothesis that coagulation of the blood is due to the formation of

¹ *Compt. rend. Soc. de biol.*, 1920, 83, 1432.

² *Jour. Infect. Dis.*, 1921, 29, 359.

³ *Arch. Dermat. and Syph.*, 1922, 5, 469.

⁴ *Lancet*, 1921, 2, 1107.

⁵ *Wien. klin. Wchn.*, 1908, 21, 214, 363.

⁶ *Biochem. Ztschr.*, 1912, xlvii, 36.

⁷ *Deut. med. Wchn.*, 1914, xl, 1607.

fibrin and fibrin is produced from fibrinogen of the plasma by the action of thrombin (fibrin-ferment). The thrombin itself consists of two substances, namely, (1) the serozyme or thrombogen, which is a protein constituent of plasma and (2) the cytozyme or thrombokinese, which belongs to the group of lipoids, particularly the lecithins, and in the blood is believed to be derived chiefly from disintegrated blood platelets. Ionized calcium must be present before these substances unite to produce thrombin, but the latter can precipitate fibrinogen into fibrin and cause coagulation in the absence of calcium ions, for example, in oxalate plasma.

While the cytozyme is derived from disintegrated platelets during the coagulation of blood, it may be secured *in vitro* by extraction from almost any cells or tissues by means of alcohol; in Hirschfeld and Klinger's test it is supplied by the ordinary alcoholic extracts of normal tissue used as antigens in the Wassermann reaction. The cytozyme or lipid material is essential in the formation of thrombin and hence in the phenomenon of coagulation; anything tending to interfere with its activity will delay or inhibit coagulation. Hirschfeld and Klinger's reaction is based upon the observation that syphilitic serum when mixed with cytozyme interferes with its activity to a greater extent than normal serum and thereby delays or prevents thrombin production and coagulation. No adequate explanation has been made of the substance in syphilitic serum responsible for this action upon the cytozyme or of the mechanism of its interference; at present syphilitic serum is regarded as possessing the property of destroying cytozyme or rendering it inactive by enmeshing the lipid particles in the globulins of the serum. Because the test is based upon the inhibition of coagulation by interference with thrombin production, the reaction has been named by Hirschfeld and Klinger the "coagulo reaction."

In conducting the test the first phase consists in mixing 0.1 or 0.2 c.c. of treated serum with 0.1 c.c. of varying dilutions of alcoholic extract of tissue (the cytozyme) and allowing the mixtures to stand for one-half to one hour to permit the inactivation or enmeshing of the lipid particles of the cytozyme by the serum, and particularly if the serum is derived from a syphilitic; calcium chlorid and serocym (fresh plasma) are then added as the second phase and the tube stood aside for fifteen minutes to permit the production of thrombin providing cytozyme is available, the amount of thrombin produced bearing a direct ratio to the amount of cytozyme present. The third phase consists in testing for the presence and amount of thrombin by adding a solution of fibrinogen and timing the reaction to determine when fibrin formation or coagulation has occurred. The controls generally coagulate within a few minutes; normal serum may delay coagulation a few minutes longer, while syphilitic serum delays coagulation for a longer period or indefinitely; the reaction, therefore, is a quantitative one. Since solutions of fibrinogen are unstable, a weak solution of oxalate plasma is employed in the last step of the test to measure the amount of thrombin present. The employment of oxalate plasma by Bordet and Delange¹ has not only the advantage of keeping for a long time, but it also prevents any further formation of thrombin from the moment at which the plasma is added, because the sodium oxalate precipitates the calcium in the form of insoluble calcium oxalate, and the consequent lack of calcium ions renders impossible the further formation of thrombin. Details of the technic will be found in the paper by the writer and Toyama² upon this reaction. We have found the reaction highly delicate and constant in syphilis, although,

¹ Ann. d. l'Inst. Pasteur, 1912, 26, 657, 737; *ibid.*, 1913, 27, 341.

² Amer. Jour. Syph., 1918, 2, 505.

in our experience, slightly less sensitive than the Wassermann reaction. Frankel and Thiele,¹ Cole and Chiu,² Nemura,³ and others have reported favorable results with this reaction.

Porges-Meier Reaction.—Porges and Meier⁴ observed that luetic serums are capable of producing flocculent precipitates from solutions of lecithin and similar salts. Two-tenths of a cubic centimeter of a 1 per cent. solution of Merck's sodium glycocholate in distilled water is placed in narrow test-tubes, and an equal amount of the patient's serum, which must be absolutely clear and inactivated by heating at 56° C. for thirty minutes, is added. This mixture and the known normal and luetic controls are kept at room temperature for from eighteen to twenty-four hours. A positive reaction is marked by the appearance of distinct coarse flocculi, mere turbidity or faint precipitation being regarded as negative.

In this connection it may be mentioned that other investigators have used other substances in similar tests, as salts of bile acids, cholesterol, vaselin, courin, palmatin, stearin, etc., but without establishing tests of practical value.

Jacobsthal⁵ has shown that by adding syphilitic serum to alcoholic extracts of tissue employed in the Wassermann test, that precipitates are formed which may be demonstrated by means of the dark-field illuminator. Bruck and Hidaka⁶ obtained similar results by macroscopic tests.

Herman-Perutz Reaction.—More recently Herman and Perutz⁷ have devised a similar test requiring the following two solutions: Solution 1 (stock solution, diluted 1 : 20 with distilled water before use) consists of: Sodium glycocholate, 2 gm.; cholesterol, 0.4 gm.; 95 per cent. alcohol, 100 c.c. Solution 2 (freshly prepared before use) is a 2 per cent. solution of sodium glycocholate in distilled water. The test is performed by adding to 0.4 c.c. of clear inactive serum (heated at 56° C. for half an hour) in a small test-tube 0.2 c.c. of solution 1 and 0.2 c.c. of solution 2. The tubes are tightly plugged with cotton and set aside at room temperature for twenty-four hours, after which the presence or absence of precipitation is noted. It is well in this test, as in all immunologic reactions, to prepare controls with known normal and luetic serums and with distilled water.

Meinicke's Reactions.—Meinicke⁸ has described three reactions based upon the hypothesis that the colloids of alcoholic extract of tissues disturb the isotonicity of saline solution permitting the union of serum globulins and lipoids. This reaction is greater in syphilitic than in non-syphilitic sera. Meinicke has described a water method, a salt solution method, and a third modification, the latter being mostly employed at the present time; the technic is as follows⁹:

The antigen is prepared from horse heart by grinding fat-free muscle and drying at 50° to 55° C. Ether is added to the powder in the proportion of 9 parts to 1 and shaken for one hour. The ether is then filtered off and the material dried at 37° C. Alcohol (96 per cent.) is then added to the powder in the proportion of 9 to 1, shaken from time to time for a day, and filtered. The filtrate is permitted to stand for several days, when it is ready for titration with alcohol and distilled water for determining the optimum combination of extract and alcohol to give the proper opalescence for tests:

¹ Münch. med. Wchn., 1914, lxi, 2095.

² Archiv. Int. Med., 1915, 16, 880.

³ Amer. Jour. Med. Sci., 1917, 154, 533.

⁴ Wien. klin. Wchn., 1908, 31, 831.

⁵ Münch. med. Wchn., 1910, 13, 41.

⁶ Ztschr. f. Immunitätsf., 1910-11, 8, 476.

⁷ Med. Klin., 1911, 2, 60.

⁸ Münch. med. Wchn., 1918, xlv, xlix. li.

⁹ Münch. med. Wchn., 1919, No. 33, 932.

TUBE.	ANTIGEN, C.C.	96 PER CENT. ALCOHOL, C.C.	DISTILLED WATER, C.C.	AFTER ONE HOUR ADD 3.5 C.C. WATER TO EACH TUBE.
1.....	0.4	0.1	0.25	Precipitation.
2.....	0.3	0.2	0.25	Very slight precipitation.
3.....	0.2	0.3	0.25	Opalescent.
4.....	0.1	0.4	0.25	Faintly opalescent.

In the above titration Tube No. 3 showed the correct combination with the particular extract employed.

After this titration seven times the quantity of 2 per cent. sodium chlorid solution is added to the mixture of extract, alcohol and water, as, for example, 0.2 c.c. extract + 0.3 c.c. alcohol + 2.5 c.c. water, and after one hour + 21 c.c. of 2 per cent. saline solution.

In conducting the test 0.8 c.c. of the diluted antigen and 0.2 c.c. of serum heated at 55° C. for twenty minutes, are mixed and left at 37.5° C. for twenty-four hours, when the readings are made. A check reading may be made after the tubes have stood an additional twenty-four hours at room temperature. In my laboratory Strumia found that this additional period of incubation improved the results by removing some doubtful reactions.

Levinson¹ states that the third modification reaction of Meinicke agrees with the Wassermann reaction in about 88.8 per cent. of sera; similar results have been reported by Stern.² Not infrequently, however, the reactions are very weak and the prolonged incubations and numerous bacterial contaminations render the readings undependable.

The Vernes Reactions.—The studies of Vernes³ upon flocculation of colloids by sera have been particularly thorough and fruitful, two tests having been elaborated for the serum diagnosis of syphilis. This investigator first worked with colloidal suspensions of inorganic substances and particularly of ferric hydrate, finding that in syphilis the serum acquires an enhanced power of flocculation and precipitation. This property of the serum in syphilis was found to fluctuate, but repeated tests at intervals generally showed a curve of flocculation higher than that shown by the serum of a non-syphilitic individual. Later on Vernes adopted a colloidal suspension of organic substances for his test in the form of a specially prepared extract of dried horse heart muscle designated as "perethynol." This extract is now employed for conducting his *direct method* and a diaphanometric scale is provided for the more accurate reading of the degree of flocculation.

The Vernes *indirect method* is a later development based upon a measure of the amount of flocculation according to the degree of inhibition of hemolysis of sheep corpuscles by swine-serum. According to Vernes, fresh swine-serum contains a substance inhibiting flocculation by syphilitic serum and a second hemolytic complex for sheep corpuscles (presumably a natural hemolysin and complement); these two agents are supposed to be bound together so that when one is exhausted the second or hemolytic substance will also be exhausted. Therefore, in mixtures of perethynol, syphilitic serum and swine-serum colloidal flocculation by the former is inhibited by the anti-flocculating substance in the latter, but the exhaustion of this substance also

¹ Amer. Jour. Syph., 1921, 5, 414.

² Ztschr. f. Immunitätsf., 1921, 32, 167.

³ Compt. rend. Acad. d. Sci., 1917, 165, 769; *ibid.*, 1918, 166, 575; *ibid.*, 1919, 167, 500; Atlas de Syphilimétrie, Boll, Paris, 1920.

removes the hemolytic activity of the swine-serum. The end-result, therefore, is an inhibition of hemolysis as occurs in the complement-fixation reaction, and Vernes believes that the degree of inhibition of hemolysis affords a more accurate measure of the flocculating power of the serum in syphilis than is possible in the direct method. By an extensive series of investigations Vernes has worked out the intricate quantitative relationships and devised a flocculation test by which the degree of flocculation by normal serum is measured; by the same technic the degree of flocculation by syphilitic serum



FIG. 145.—A SOXHLET EXTRACTION APPARATUS WITH VACUUM.

is also measured and plotted into curves (syphilimetry). He noted that normal sera gave a horizontal line, but in syphilis the curve of flocculation oscillates up and down during the course of weeks or months.

Antigen.—This is the same for both methods and is prepared by successive distillations under negative pressure with the perchlorid of ethylene and alcohol; it is named "perethynol" and is prepared as follows¹:

¹ Bull. d. Sc. Pharmacol., 1918, 25, 321.

1. Secure fresh horse heart and grind the muscle to a pulp. Dehydrate by adding several volumes of 95 per cent. alcohol and allow to stand for one hour; express the alcohol and repeat. Express the alcohol, spread the material on glass plates, and dry at 37° C. for twenty-four hours. Grind the material into a fine powder.

2. To 30 gm. of powdered muscle in a 500 c.c. distilling flask add 60 gm. of washed and dried sand and 250 c.c. of ethylene perchlorid with a boiling-point of 115° to 121° C. Connect with a Soxhlet extracting and condensing apparatus (Fig. 145) in such way that the distillation is conducted under a partial vacuum by means of an air or water pump. The distilling flask is heated in a water-bath at a temperature of 60° to 65° C. and with a pressure of 4 cm. of mercury, so that the temperature in the distilling flask does not exceed 35° C. This requires from six to seven hours.

3. The distillate is discarded, the residue again dried at 37° C., and again extracted for five hours with 200 c.c. of absolute ethyl alcohol in the same apparatus at a pressure of 5 to 6 cm. of mercury. The temperature of the water-bath should be 60° to 65° C. and the contents of the flask about 30° C.

4. The residue is discarded. The distillate is allowed to stand for twenty-four hours and filtered. About 25 c.c. are dried in a weighed dish at 60° C. and weighed. On the basis of this calculation the alcoholic extract is adjusted so that it contains 0.15 gm. dried extract per 1000 c.c. If it contains more than this amount, add the necessary amount of absolute ethyl alcohol; if less, the extract must be concentrated to the necessary degree by evaporation in a vacuum at 30° C.

In the *direct method* the antigen (perethynol) is diluted by adding 1 part of antigen (perethynol) drop by drop to 6.5 parts of water. The serum is heated thirty minutes at 55° C. and 0.8 c.c. added to 0.4 c.c. of the antigen. This mixture is allowed to stand for twenty-four hours at room temperature (19° to 22° C.) and centrifuged. The superfluid is removed and the sediment shaken up in 2.4 c.c. of doubly distilled water slightly charged with carbonic acid and the amount of flocculation recorded by comparison with a diaphanometric scale prepared by a mixture of water and tincture of benzoin. Spontaneous precipitation is prevented by the use of glycerin and tincture of quillaja as disseminating agents. The readings are made over a black inclined background with diffuse light or electric arc.

The scale is prepared as follows: Mix 250 c.c. of tincture of benzoin, 125 c.c. tincture of quillaja, and 250 c.c. of 80 per cent. alcohol.

Add 10 c.c. of this mixture drop by drop to 50 c.c. of glycerin-water (glycerin 30 c.c. plus water 70 c.c.), while constantly stirring. This suspension is further diluted one-fourth with the 30 per cent. glycerin-water and distributed in the following amounts in a series of 10 test-tubes of uniform caliber (13 mm. outside) each containing 2 c.c. of 50 per cent. glycerin-water: 0.01 (No. 1), 0.015, 0.0225, 0.0337, 0.0405, 0.0606, 0.0909, 0.1363, 0.2194, and 0.3291 c.c. (No. 10). Each tube contains 1.5 times the dose in the preceding tube.

The *indirect method*, which is more generally employed, is conducted as follows¹:

The *extract* (perethynol) is diluted 1 : 40 with 0.9 per cent. saline solution. The required amount of saline is placed in a beaker and while being mechanically stirred by means of a small glass propeller attached to an electric motor (Fig. 146) at the rate of about 300 revolutions per minute, the extract is added drop by drop in such manner as to avoid touching the side of the beaker. The suspension should be freshly prepared just previous

¹ Compt. rend. Acad. d. Sci., 1918, 167, 385, 500; *ibid.*, 1919, 168, 247.

to the titration and main tests and kept constantly stirred until distributed in the tubes. The suspension should correspond to tube 5 of the diaphanometric scale described above.



FIG. 146.—AN ELECTRICALLY DRIVEN MIXER FOR PREPARING PERETHYNOL SUSPENSION FOR VERNES' TEST.

The *sheep corpuscles* are washed three times with hypertonic saline solution prepared as follows:

Sodium chlorid.....	9.5 gm.
Sodium bicarbonate.....	0.15 "
Potassium chlorid.....	0.42 "
Calcium chlorid.....	0.125 "
Distilled water.....	1000 c.c.

After the last washing the packed cells are made up into a 50 per cent. suspension by adding an equal volume of the hypertonic saline solution.

The suspension is then titrated by placing the following amounts in six test-tubes: 0.025, 0.05, 0.075, 0.1, 0.125, and 0.15 c.c.; distilled water is added to make the total volume in each tube exactly 2.6 c.c. as follows: 2.575, 2.55, 2.525, 2.5, 2.475, and 2.45 c.c. The proper dose of cells for the tests is the amount corresponding to the color of this mixture:

Acid fuchsin (Grubler) 0.1 per cent. in distilled water.....	1.0 c.c.
Picric acid 1 per cent. in distilled water.....	1.0 "
Glacial acetic acid.....	0.45 "
Formaldehyd solution (40 per cent.).....	0.25 "
Distilled water.....	10.0 "

The color given by this solution corresponds to Tint 8 and is called Solution 8; other solutions are prepared from it by diluting with this solution (glacial acetic acid, 4.5 c.c.; formaldehyd solution (40 per cent.), 2.5 c.c., and distilled water 100 c.c.) as follows in test-tubes:

No. 7: 2 c.c. of No. 8 + 2 c.c. diluent (Tint 7).

No. 6: 1 c.c. of No. 8 + 2 c.c. diluent (Tint 6).

No. 5: 2 c.c. of No. 8 + 7 c.c. diluent (Tint 5).

No. 4: 0.4 c.c. of No. 8 + 2.3 c.c. diluent (Tint 4).

No. 3: 0.8 c.c. of No. 8 + 7.3 c.c. diluent (Tint 3).

No. 2: 1.6 c.c. of No. 8 + 22.7 c.c. diluent (Tint 2).

No. 1: 0.32 c.c. of No. 8 + 6.97 c.c. diluent (Tint 1).

No. 0: 0.1 c.c. of No. 8 + 6.4 c.c. diluent (Tint 0).

About 3 c.c. of each of these solutions should be placed in scrupulously cleaned and dry test-tubes of non-sol-glass having a uniform caliber (13 mm. outside), stoppered with cork, properly labelled 8 to 0, and kept in a dark place.

Sufficient suspension for the tests to be conducted is made up with the hypertonic saline in such manner that the dose is contained in 0.6 c.c.; example:

Dose of cells, 0.075.

Number of doses required, 200; prepared by diluting 15 c.c. of the 50 per cent. suspension with 105 c.c. of the hypertonic saline solution; dose, 0.6 c.c.

The *swine-serum* may be obtained by collecting blood in a vessel in an abattoir and allowing the serum to separate. It must be fresh in order to preserve the complement and natural antishcep hemolysin and must be titrated each time for (a) hemolytic activity as well as for (b) the inhibiting influence of perethynol and (c) the proper adjustment of the albumin content. Not all swine-sera are satisfactory; some are lacking in sufficient complement, hemolysin, or both. *A mixture of several should be used* and blood may be obtained from an abattoir. The titrations are conducted as follows:

(a) Arrange two series of 11 test-tubes of regulation size.

(b) Place 5 c.c. swine-serum in a small beaker and dilute 1 : 3.2 by adding 11 c.c. of saline solution; mix well. Remove and discard exactly 1.5 c.c., leaving a balance of 10 c.c.

(c) Place 0.8 c.c. of this dilution in the first tube of each series. Place 0.8 c.c. of saline solution in the beaker and mix well (avoid bubbling). Then place 0.8 c.c. in the second tube of each series. Place 0.8 c.c. of saline solution in the beaker and mix well. Then place 0.8 c.c. in the third tube of each series and continue in this manner until each of the 11 tubes of the two series have received 0.8 c.c. of diluted swine-serum.

(d) In each tube of the first series (A) place 1.2 c.c. of 0.9 per cent. saline solution and 0.6 c.c. (the dose) of sheep cell suspension. Mix well and incubate in a thermostat at 38° C. for twenty-five minutes. Centrifuge the tubes showing hemolysis above Tint 5.

(e) In each tube of the second series (B) place 1.2 c.c. of the perethynol suspension. Mix well and incubate at 38° C. for forty-five minutes. Add 0.6 c.c. of sheep cell suspension, mix, and reincubate for twenty-five minutes; centrifuge the tubes showing hemolysis above Tint 5.

(f) It is necessary to accurately determine the points of complete hemolysis in both series, and for this reason all tubes showing hemolysis above Tint 5 are centrifuged. Pour off the superfluids carefully and completely and add 2.4 c.c. of hypertonic saline to each, mix well, and centrifuge. Pour off the superfluids and add 2.4 c.c. of distilled water to each tube; mix well. Hemolysis will now occur and thereby show the presence of corpuscles not completely hemolyzed in the titrations. In this manner the smallest amounts of pig-serum required for complete hemolysis in both series may be accurately determined.

(g) According to the method of dilution employed the tubes of each series contain the following amounts of *undiluted swine-serum*:

Tube	1	=	0.250	c.c.
"	2	=	0.228	"
"	3	=	0.206	"
"	4	=	0.185	"
"	5	=	0.163	"
"	6	=	0.161	"
"	7	=	0.120	"
"	8	=	0.098	"
"	9	=	0.054	"
"	10	=	0.033	"
"	11	=	0.003	"

The dose of serum required for the test is obtained by dividing the smallest completely hemolytic dose in *B* by the smallest completely hemolytic dose in *A*, and is usually between 0.15 and 0.25 c.c.

If the dose is less than 0.18 c.c. add sufficient pig-serum heated at 55° C. for thirty minutes to make the dose 0.2 c.c. This is for the purpose of maintaining the albumin content at an optimum point. If below this point flocculation is considerably increased, thereby utilizing to a greater extent the antiflocculent property of pig-serum and consequently displacing the syphilitic index toward the positive phase (Tint 0). It is unnecessary to make any correction for the slight difference between 0.18 and 0.2 c.c.

(h) All of the manipulations in this titration and in the main tests should be made within a constant time limit and at a uniform temperature. The optimum temperature is 20° C. If the temperature of the laboratory is above or below 20° C., corrections in the dose of complement should be made as follows:

- 16° C. subtract 0.02 c.c. from the dose.
- 17° C. subtract 0.015 c.c. from the dose.
- 18° C. subtract 0.01 c.c. from the dose.
- 19° C. subtract 0.005 c.c. from the dose.
- 21° C. add 0.005 c.c. to the dose.
- 22° C. add 0.01 c.c. to the dose.
- 23° C. add 0.015 c.c. to the dose.
- 24° C. add 0.02 c.c. to the dose.

The swine-serum is now diluted with 0.9 per cent. saline solution so that the dose is 0.8 c.c. Example:

Hemolytic unit of Series B = 0.287 c.c. (Tube 3).

Hemolytic unit of Series A = 0.137 c.c. (Tube 8).

$$\text{Dose} = \frac{0.2064}{0.098} = 0.210 \text{ c.c.}$$

Temperature of laboratory 22° C.

Corrected dose: $0.210 + 0.01 = 0.220 \text{ c.c.}$

200 doses of 0.8 c.c. required = 43.8 c.c. serum + 116.2 c.c. saline.

Main Test.—(a) The sera should be fresh, free of corpuscles, and heated to 55° C. for twenty minutes.

(b) For each serum to be tested arrange 2 regulation test-tubes and place 0.2 c.c. serum in each.

(c) Spinal fluids are used unheated; dose in each tube, 1.6 c.c.

(d) To the first tube of each set add 0.8 c.c. of perethynol diluted 1 : 40 with 0.9 per cent. saline solution; to the second tube add 0.8 c.c. of saline solution (controls).

(e) To each tube carrying serum add 0.8 c.c. diluted swine-serum carrying the proper dose. With spinal fluids use proper dose of undiluted swine-serum in order not to increase the volume.

(f) Place the tubes in a thermostat at 38° C. for seventy-five minutes if the temperature of the laboratory is 12° to 15° C., or for sixty minutes if the temperature is 18° to 22° C.

(g) Add 0.6 c.c. of sheep cells in appropriate dilution; mix and reincubate for twenty to twenty-five minutes. When the serum controls are hemolyzed, the front tubes containing perethynol are centrifuged and the tints of supernatant fluids compared with the color scale and recorded.

Readings.—A normal non-syphilitic serum should give Tint 8 of complete hemolysis. Any hemolysis less than this indicates a positive reaction. A complete positive would be Tint 0 (no hemolysis) and partial hemolysis is represented by the intermediate tints.

Vernes claims that these methods possess a high degree of practical value in the diagnosis of syphilis and as a serologic guide to therapy. Owing principally to difficulties in technic it has not been extensively employed by others. Cornwall¹ found that in cases of syphilis under treatment the spinal fluid gave positive reactions with the Vernes test in about 12 per cent. more cases than with the Wassermann test. With sera, however, the Wassermann reaction was positive in about 19 per cent. more cases than the Vernes reaction.

Vernes regards the reaction a better guide to the treatment of syphilis than the Wassermann reaction. While it is claimed to be a different mechanism, yet the indirect method appears to be a complement-fixation reaction in which the complement and hemolysin is supplied by active swine-serum. Very probably the Wassermann and Vernes reactions are identical colloidal phenomena, and in my experience the latter reaction has not yielded better results either in the diagnosis or treatment of syphilis than a quantitative complement-fixation test.

Sachs-Georgi Reaction.—At the present time considerable interest is being given a flocculation reaction described by Sachs and Georgi² employing cholesterolized alcoholic extract of heart muscle.

The *preparation of the extract* appears to be an important element in the test and already numerous modifications have appeared. Beef heart muscle is passed through a meat grinder and 25 gm. ground with sand and extracted with 125 c.c. of 95 per cent. alcohol by vigorous shaking in a machine for six hours. It is then placed in an incubator over night, filtered through paper and stored in a refrigerator for several days followed by refiltration. To 100 c.c. of the filtrate add 200 c.c. of 95 per cent. alcohol and 18 c.c. of a 1 per cent. solution of pure cholesterol in alcohol (0.6 per cent. cholesterol).

Sachs and Georgi state that most of their extracts have been satisfactory

¹ Arch. Dermat. and Syph., 1922, 5, 433.

² Med. Klinik., 1918, No. 33, 805; Münch. med. Wchn., 1920, 67, 66.

with the addition of 0.045 to 0.06 per cent. cholesterol. Parker and Haigh¹ have used 0.06 to 0.07 per cent. solutions and advise a preliminary titration to determine the maximum amount to employ. This may be accomplished by placing 3 c.c. of the alcoholic antigen (100 c.c. extract + 200 c.c. alcohol) in each of 5 test-tubes and adding the following amounts of 1 per cent. alcoholic solution of cholesterol: 0.135, 0.15, 0.18, 0.21, and 0.24 c.c., the percentages being respectively 0.045, 0.05, 0.06, 0.07, and 0.08. Each of these five mixtures are then employed in tests with normal and syphilitic sera as described below, and if possible with duplicate tests set up with an extract of known properties.

The *serum* should be fresh, free of corpuscles, heated to 55° C. for half an hour, and allowed to cool for three hours, as Munster has shown that sera used immediately after heating may yield doubtful reactions. Spinal fluid is used unheated.

The *test* is conducted as follows: The *extract is diluted 1 : 5* and the method of dilution is an important matter. The required amount of extract is placed in a small Erlenmeyer flask and an equal amount of saline solution rapidly added. The mixture is gently shaken and allowed to stand ten minutes, when the balance of the 4 volumes of saline is rapidly added. The mixture is again shaken gently and is ready for use. Example: 10 c.c. extract + 10 c.c. saline solution; mix, stand ten minutes, add 30 c.c. saline solution, and mix. Craig and Williams² have found that much better results were observed when the saline solution was added to the extract drop by drop and the mixture allowed to stand for two hours before use.

The *test* is conducted as follows:

(a) For each serum and spinal fluid arrange two small test-tubes (outside diameter 13 mm.).

(b) Place 0.1 c.c. serum and 0.9 c.c. saline solution in each tube. With spinal fluids place 1.5 c.c. undiluted in each tube.

(c) To the first or front tubes of each serum test add 0.5 c.c. of the diluted extract; in spinal fluid tests add 0.75 c.c. of extract to each front tube.

(d) To the second or rear tubes add 0.5 c.c. of a 1 : 5 dilution of 95 per cent. alcohol in saline solution. These are the serum controls, and if precipitates form the serum is unsuitable.

(e) In an extra tube place 0.5 c.c. of the diluted extract and 1 c.c. saline solution (antigen control).

(f) All tubes are thoroughly shaken and placed in an incubator at 37.5° C. for two hours and then allowed to stand at room temperature for eighteen to twenty hours, when the reaction may be read. Neykirch³ advises twenty hours at 37.5° C. followed by twenty hours in a refrigerator in order to bring out the weakly positive reactions and permit non-specific precipitates to disappear.

(g) Meyer⁴ has shown that the reactions may be read by centrifuging the tubes; Hull and Faught⁵ state that if the tests are centrifuged immediately after adding the extract, they can be read as well as after standing twenty-four hours. Centrifuging is often of aid in weakly positive reactions followed by shaking when compact flocculi rise from the bottom of the tube.

¹ Arch. Dermat. and Syph., 1921, 4, 67.

² Jour. Amer. Med. Assoc., 1922, 79, 1597.

³ Arb. a. d. Inst. f. Exper. Ther. u. G. S. H., 1920, 10, 49.

⁴ Berl. klin. Wchn., 1919, No. 14, 331.

⁵ Jour. Immunology, 1920, 5, 521.

(h) Readings may be made with the naked eye or with the aid of a reading glass against a slanted black background and recorded as follows:

++++ (Strongly positive): heavy, flocculent precipitate and clear supernatant fluid.

+++ (Moderately positive): diffuse, finely flocculent precipitate; supernatant fluid contains flocculi.

++ (Weakly positive): slight amount of precipitate.

— (Negative): no flocculation. A slight grayish sediment may be found, which, on shaking, is dispersed in the supernatant fluid.

A large literature has already accumulated upon the results observed with this test as compared with the Wassermann reaction. Sachs and Georgi,¹ in a review of the literature covering 12,124 tests, found an agreement in 92.44 per cent. In 3 per cent. the Wassermann reaction was positive or doubtful and the Sachs-Georgi reaction negative; in approximately 7 per cent. the Wassermann reaction was negative and the Sachs-Georgi reaction positive. Messerschmidt² reported 1100 comparative Wassermann and Sachs-Georgi reactions with an agreement in 85 per cent., Kirschner and Segall³ on over 1000 similar tests, with an agreement of 80 per cent., and Baumgartel⁴ on 7000 tests, with 90 per cent. agreement. Favorable reports have also been made by Levinson and Petersen,⁵ Gaechtjens,⁶ D'Annoy,⁷ Harryman,⁸ Logan,⁹ Parker and Haigh,¹⁰ and others. My own experience is closely similar to that of Kilduff¹¹ and Craig and Williams,¹² who found the reactions often difficult to read, and prone to error on the negative side. I have not found the test with either serum or spinal fluid as sensitive and reliable as the complement-fixation test either for the diagnosis of syphilis or as a serologic guide to treatment.

Sigma Reaction of Dreyer and Ward.—A similar reaction has recently been described by Dreyer and Ward¹³ except that it employs nine different amounts of serum and aims to yield a quantitative reaction.

The antigen is a mixture of an acetone-free alcohol soluble heart extract and cholesterol, originally described by Bordet and Ruelens.¹⁴ The antigen is carefully diluted with saline solution to give a standard opacity and a fixed amount used with varying amounts of heated serum using a Dreyer pipet and Dreyer tubes, to give dilutions 1 : 1.25, 1 : 2.5, 1 : 5.2, 1 : 13.1, 1 : 26.4, 1 : 46, 1 : 96, 1 : 232, and 1 : 462.

The tubes are incubated in a water-bath at 37° C. for seven hours and read with the aid of a hand lens. Rook¹⁵ has reported the reaction with sera as good, or even slightly better than the results obtained by the Wassermann reaction; with spinal fluids the results were less satisfactory.

The Kahn Reaction.—Kahn¹⁶ has endeavored to improve the Sachs-Georgi test by a new extract containing more lipoids and by shortening the period of incubation; both test-tube and microscopic methods have been described.¹⁷

The *extract* is prepared of beef or guinea-pig heart after the method of Neymann and Gager¹⁸: a weighed amount of finely ground and dried heart

¹ Arb. a. d. Inst. f. Exper. Therapie, 1920, 10, 5.

² Deut. med. Wchn., 1920, No. 6, 150.

³ Wien. klin. Wchn., 1920, No. 18, 377.

⁴ Münch. med. Wchn., 1920, No. 15, 421.

⁵ Arch. Dermat. and Syph., 1921, 3, 286.

⁶ Arch. Dermat. and Syph., 1921, 129, 2.

⁷ Jour. Med. Research, 1921, xlii, 339.

⁸ Arch. Dermat. and Syph., 1921, 4, 299.

⁹ Arch. Dermat. and Syph., 1922, 5, 570.

¹⁰ Arch. Dermat. and Syph., 1922, 6, 332.

¹¹ Lancet, 1921, 1, 14.

¹² Arch. Dermat. and Syph., 1921, 4, 67.

¹³ Arch. Dermat. and Syph., 1921, 3, 415.

¹⁴ Jour. Amer. Med. Assoc., 1922, 79, 1597.

¹⁵ Lancet, 1921, 1, 956.

¹⁶ Compt. rend. Soc. de biol., 1919.

¹⁷ Lancet, 1922, 1, 118.

¹⁸ Soc. Exper. Biol. and Med., 1922, 19, 182.

¹⁹ Jour. Immunology, 1917, 2, 573.

muscle is extracted with several liberal amounts of ether in a refrigerator until supernatant ether is free from coloring matter (usually three to five days with daily change of ether). The ether is filtered off after each extraction and discarded. The heart muscle is then spread on filter-paper and dried until free from ether odor. Five cubic centimeters of alcohol (absolute or 95 per cent.) are added to each grain of powder and extracted for nine days in a refrigerator and one day at room temperature with frequent shakings. The alcohol is filtered off and a portion used as such for plain antigen; the remainder is cholesterolized as follows: to each 100 c.c. add 0.4 gm. pure cholesterol. Dissolve by warming in a water-bath with gentle rotation. Filter through several layers of filter-paper to remove impurities as well as undissolved cholesterol and allow to stand at room temperature for twenty-four hours before use.

For use the plain extract is diluted 1:2 with saline solution and the cholesterolized extract 1:3, the latter being prepared as follows: Measure 5 c.c. of antigen into a dry 25 c.c. cylinder. Place 10 c.c. of physiologic saline solution into another 25 c.c. cylinder. Pour the saline into the antigen in about the same manner as one might add physiologic saline solution to red corpuscles. Invert the mixture back and forth several times.

This mixture is opalescent and somewhat milky and may be kept in an incubator for several weeks without loss of activity. As a general rule the extract is employed about one-half hour after its dilution.

The *serum* must be fresh, clear, and *free of corpuscles*. The presence or absence of natural antishcep hemolysin has no influence. Serum is heated at 56° C. for thirty minutes. The *test* is conducted as follows:

(a) It is recommended that each serum be tested with two or three dilutions of the same extract or with two or three different extracts.

(b) In small test-tubes place 0.3 c.c. serum and add 0.05 c.c. diluted extract. Spinal fluids are used in dose of 1 c.c.

(c) Mix the contents of each tube vigorously for two or three minutes and incubate in a thermostat at 38° C. for sixteen to eighteen hours, or for four hours in a water-bath followed by overnight in a refrigerator

(d) "The results are read after about eighteen hours' incubation. The tubes should not be shaken again before the first reading. The specific precipitates are lipoidal in character and are suspended in the medium. The strongly positive will be recognized without difficulty. The ++++ reactions will show either one or several clumps. The +++ reactions will show comparatively large flocculi or granules. The ++ reactions will show clumps or granules of a lesser size, but large enough to be unmistakable. The + and ± reactions are best seen by slanting the tubes and observing the upper point of contact between the fluid and tube wall. The slanted tube should be several inches above the level of the eye and held in front of a window, focusing on some dark object, such as a window shade or frame. One will then see, by looking up, a thin layer of fluid with a precipitate floating in it."

The cholesterolized extracts are generally more sensitive than the plain alcoholic extracts, and with the eighteen-hour period of incubation great care must be exercised in the interpretation of weakly positive reactions.

With about 80 per cent. of strongly positive Wassermann reactions a precipitate may appear within five minutes after the addition of antigen to the serum followed by vigorous shaking for several minutes. Kahn has also described a micro-method employing 0.03 c.c. of serum and 0.005 c.c. of diluted antigen. Herrold¹ has recently described a modification embrac-

¹ Jour. Amer. Med. Assoc., 1922, 79, 957.

ing a ring reaction at the line of contact between serum and antigen. In my laboratory this method proved inferior to the test described above.

The true precipitate is whitish and floats in the fluid and is best seen by merely slanting the tubes and observing the upper layer of fluid. Shaking may bring out dust particles and spontaneous serum precipitates and suggests doubtful reactions. By adding 0.8 c.c. saline solution to such tubes, true precipitates are visible, while pseudo or serum precipitates disappear.

Of 1119 comparative tests carried out with the precipitation and Wassermann reactions, Kahn found 98 per cent. to agree in results, and he hopes that this test will form an important supplement to the Wassermann test.

Keim and Wile¹ have employed the test in the study of 350 sera with satisfactory results, believing that its simplicity, rapidity of reading and reduction of sources of error constitute advantages over the Wassermann reaction. Ide and Smith² have employed the test with 2165 sera and believe that it is of value as a check on the Wassermann reaction. They have also reported that the test yields satisfactory results with sera proving anti-complementary in the complement-fixation reaction. In my laboratory Strumia has studied this and other precipitation reactions in syphilis, and found that with sera yielding strongly positive or negative complement-fixation reactions the reactions with both tests were closely parallel, but with sera containing small amounts of syphilis "reagin," as in latent tertiary cases of syphilis and cases under treatment, that the complement-fixation test was generally superior in sensitiveness and practical value.

MECHANISM OF PRECIPITATION IN SYPHILIS AND RELATION TO COMPLEMENT FIXATION

Numerous investigations upon the mechanism of the various flocculation reactions in syphilis have shown that chemical changes occur mainly involving the globulins of the serum and that flocculation is apparently a physico-chemical phenomenon involving the interaction of substances in colloidal suspension. The important relation of electrolytes and particularly of sodium chlorid to the phenomenon as shown, especially by Meinicke, have indicated the colloidal nature of these reactions, as is true of serum agglutination and precipitation in other diseases as well.

Reference has already been made to the increase of serum globulins occurring in syphilis, probably a result of irritation of body cells by the products of *Treponema pallidum*. The water reaction of Klausner, the nitric and lactic acid tests of Bruck with serum, and the butyric acid test of Noguchi with spinal fluid are based upon this primary change, but at the present time none of these or similar tests evolved by others have proved sufficiently quantitative or differential for diagnostic purposes. Furthermore, the mere increase of serum globulins in syphilis does not necessarily explain or bear a relation to the colloidal flocculation and complement-fixation reactions, but simply represents an additional physical change apart from the chemical changes concerned in these reactions.

Normal human serum is capable of flocculating various inorganic and organic substances in colloidal suspensions as shown by Lange, of colloidal iron as shown by Vernes, and of mastic, benzoin, and various lipoidal substances in colloidal suspension as shown by the numerous flocculation tests devised for the serum diagnosis of syphilis beginning with the work of Michaelis over fifteen years ago. But in syphilis this power of serum and spinal fluid for precipitating inorganic and organic colloids is increased, and

¹ Jour. Amer. Med. Assoc., 1922, 79, 870.

² Arch. Dermat. and Syph., 1922, 6, 770.

usually in a measurable degree; or the serum proteins and especially the globulins acquire in syphilis an increased sensitiveness to flocculation by various organic and inorganic colloids.

Chemical studies of the flocculi or precipitates produced by various flocculation tests have generally shown the presence of both serum proteins (especially globulins) and substrate. Two theories or hypotheses may be advanced, therefore, in explanation of these colloidal precipitating reactions as follows:

(a) That in syphilis the colloidal serum proteins and especially the globulins acquire an increased sensitiveness to flocculation or precipitation by other colloids and especially lipoidal substances contained in the "antigen" (alcoholic tissue extracts) or,

(b) That in syphilis a new and foreign antibody-like substance is produced, associated or linked with the globulins of the serum, possessing a greater power for flocculating organic (tissue lipoids) and inorganic (iron, gold, etc.) colloids in suspension, than possessed by the serum proteins of healthy individuals.

Available data indicate that the latter or second hypothesis is more tenable than the former, and that an antibody-like substance or cellular reactionary agent is produced and linked or associated with the globulins of the serum as is true of antibodies in general. Because this "reagin" is capable of flocculating lipoids in colloidal suspension it is commonly believed that it may be a lipoid-protein compound, but its exact chemical nature has not been determined.

There can be no doubt, however, that the flocculating substance produced in syphilis and present in the blood and spinal fluid has a special effect upon lipoidal substances in colloidal suspension, and for this reason the substrate for most flocculating tests is an alcoholic extract of some tissue. The methods of Herman and Perutz, Porges, and Meier, Meinicke, Sachs and Georgi, Vernes, Kahn, etc., employ substrates of this kind or solutions of isolated lipoids, and the occurrence, sensitiveness, and practical value of the reactions in syphilis depend to a remarkable degree upon the method of preparing and diluting the extracts, as is true of the "antigens" or extracts employed in the Bordet-Wassermann reaction. In the coagulation test of Hirschfeld and Klinger, a tissue lipoidal extract (the cytozyme) is regarded as essential for the production of thrombin by interaction with a serozyme (an albuminoid) and a calcium ion, and the reaction is based upon the observations that syphilitic serum inhibits coagulation by its effect upon the lipoidal extract (cytozyme), probably one of inactivation by flocculation.

Why this syphilis "reagin" or antibody-like substance has this flocculating effect upon lipoids in colloidal suspension is unknown. Some investigators believe that it is an antibody for lipoids produced in syphilis; that in this disease as well as in frambesia, a foreign lipoidal antigen is engendered through the action of the products of spirochetes upon body cells which is capable of calling forth a peculiar lipodophilic antibody or reagin. The weight of evidence is against the assumption that protein free lipoids can engender the production of antibodies, but the stimulating substance may be a lipo-protein compound produced by cells irritated by these pathogenic spirochetes and capable of engendering the production of an antibody or reactionary product of unknown chemical nature causing the flocculation or precipitation of organic and inorganic colloid in the test-tube under favorable conditions.

In my opinion the fundamental mechanism of complement fixation in syphilis is identical with these macroscopic colloidal flocculation reactions. There is no important or essential reason for believing otherwise. The

agglutination and precipitin reactions with formed elements and proteins in solution are colloidal reactions even though the chemical nature of the antibody in the serum is unknown. It is entirely reasonable to assume that in the Bordet-Wassermann reaction the same "reagin" or antibody-like substance is operative as in the various flocculation reactions; that in the former flocculation occurs which is invisible to the naked eye, but sometimes visible microscopically by the dark-field illumination method. In a general manner the tissue extracts yielding best results in the complement-fixation reaction also prove most sensitive in the macroscopic flocculation reaction, *e. g.*, the cholesterolized alcoholic extracts. The degree of dilution, however, varies for the two methods, but the manner of diluting the extracts with saline solution is essentially the same for both.

Just what happens to the complement or alexin is unknown. It is probably fixed or absorbed by the precipitate and its hemolytic activity inactivated. However, it is possible that the complement serum has two qualities closely linked, one that is hemolytic and the other antiflocculating; that in mixtures of syphilitic serum and lipoidal extracts the antiflocculating substance is consumed in counteracting the flocculation of proteins and lipoids in colloidal suspension, and that this removes at the same time the hemolytic complex. Vernes has advanced this theory in explanation of the rôle of fresh swine-serum in his indirect method, but whatever may be the true explanation, I believe that the indirect method of Vernes is nothing more than a complement-fixation reaction utilizing the complement and natural antishoop hemolysin of swine-serum. At any rate, I have conducted his test with equally good results employing the usual guinea-pig complement and rabbit antishoop hemolysin.

SPECIFICITY AND PRACTICAL VALUE OF PRECIPITATION REACTIONS IN SYPHILIS

Originally studied in relation to the mechanism of complement fixation in syphilis, flocculation or precipitation tests have since been advocated as practical means for the serum diagnosis of this disease. Methods evolved from time to time and compared with the Wassermann reaction were soon found to be inferior as practical diagnostic methods, but during the past few years the methods of Meinicke, Vernes, Sachs and Georgi, and Kahn have commanded much attention with the accumulation of a large literature, especially upon the Sachs-Georgi reaction.

Advantages of Precipitation Tests.—As compared with the technic of the complement-fixation test, the precipitation methods are *simpler*, and this constitutes their main advantage. They are likewise more economical, since sheep corpuscles, hemolysin, and complement are not employed. They do not require preliminary titrations of complement and hemolysin, but, on the other hand, the preparation and titration of the extract employed as "antigen" requires as much attention as in the complement-fixation test. Contrary to current opinion the results of flocculation reactions are greatly influenced by technical conditions and especially the manner of diluting and using the extract, temperature and duration of incubation of the mixtures of extract and serum, etc.; furthermore, considerable experience is required in the reading and interpretation of the reactions, probably more than required for complement-fixation tests. Owing to the simplicity of the technic when extract is available, it would appear off hand that one of these methods may prove of value as a substitute for the complement-fixation test for the serum diagnosis of syphilis on ships and under similar conditions in which the latter test cannot be conducted, but in my opinion they cannot

be relied upon *alone* for the serum diagnosis of clinically obscure or doubtful cases of syphilis or as a serologic guide to treatment.

Disadvantages of the Precipitation Tests.—From the technical standpoint, many reactions are *difficult to read and interpret*. With serums yielding ++++ complement-fixation reactions, flocculation is usually well marked, but with serums containing less "reagin" the degree of precipitation is frequently slight and readily overlooked or confused with non-specific reactions. My experience in this regard is similar to that of Craig and Williams¹: "The difficulty of reading slight Sachs-Georgi reactions and differentiating them from precipitation due to other causes in the serum is so great, in many instances, that no reliable conclusion can be reached, thus leaving the test open to so much individual interpretation that it destroys the scientific value of the results."

Considerable experience is required for reading the reactions—more, in my estimation, than required by a complement-fixation reaction.

From the biologic standpoint precipitation methods are less sensitive than acceptable complement-fixation tests and probably more subject to the error or falsely positive reactions, despite their simpler technic.

Sensitiveness of the Precipitation Reactions.—Most investigators have based their opinion on the value of flocculation reactions in syphilis according to comparative results with the Wassermann reaction or one of its modifications, and needless to state the sensitiveness and syphilitic specificity of the latter have influenced the percentages of positive and negative reactions and the opinions based upon these. My analysis of the literature covering over 24,000 comparative tests employing the Meinicke (third modification), Sachs-Georgi, and Kahn reactions, have shown that the positive and negative results agreed with complement-fixation reactions in from 80 to 95 per cent. of serums with a general average of about 89 to 90 per cent. About 8 per cent. of the differences were due to positive complement-fixation and negative flocculation reactions and 3 per cent. to negative complement-fixation and positive flocculation reactions.

The results observed by Strumia, in a comparative study of several flocculation tests with my new complement-fixation test for syphilis based upon studies in the standardization of technic, have shown the following results:

COMPARISON OF COMPLEMENT-FIXATION AND FLOCCULATION REACTIONS IN SYPHILIS

FLOCCULATION TESTS.	SERA TESTED.	COMPLEMENT-FIXATION REACTIONS.		FLOCCULATION REACTIONS.			AGREEMENT.
		Per cent. positive.	Per cent. negative.	Per cent. positive.	Per cent. negative.	Per cent. doubtful.	
Meinicke (third modification) . . .	705	40.4	59.6	39.5	58.7	1.7	89.2
Sachs-Georgi	645	40.3	59.7	47.8	50.3	1.9	85.3
Kahn (cholesterolized antigen) . .	624	41	59	49.8	48.2	2	83.4
Kahn (plain antigen)	566	40	60	30.4	65.5	4	82.7
Vernes (direct method)	503	37.6	62.5	44.1	55.5	0.4	89.3
Vernes (indirect method)	500	38.4	61.6	41.6	56.6	1.8	90

With the serums of the majority of syphilitic individuals the flocculation reactions are positive and practically specific, but with about 8 to 15 per

¹ Jour. Amer. Med. Assoc., 1922, 79, 1597.

cent. of serums from individuals with latent and tertiary syphilis and containing small amounts of "reagin" the tests yield falsely negative reactions. Our aim should be to improve the sensitiveness of complement-fixation and flocculation reactions, because even under the best of conditions the reactions are still too frequently negative in syphilis. As the matter stands at present a good, sensitive complement-fixation reaction is our best means for the serum diagnosis of syphilis, and especially syphilis in its clinically difficult and unrecognizable stages. As stated by Craig and Williams, no less than 33.3 per cent. of the positive Wassermann reactions were missed by the Sachs-Georgi test in known cases of syphilis, while 26.5 per cent. of the positive Sachs-Georgi reactions were not confirmed by the Wassermann test nor by clinical findings.

Others have observed better results—with the Sachs-Georgi and Kahn methods the reactions have agreed with the complement-fixation reaction in as high as 95 to 98 per cent. of serums, but the significance of these figures is reduced unless the complement-fixation test is known to be sensitive and reliable and the numerous modifications of the Bordet-Wassermann reaction in use are known to vary in these regards.

As is to be expected in any large series of comparative tests, the flocculation methods sometimes have yielded positive reactions in primary syphilis before the complement-fixation test has become positive, and likewise have yielded positive reactions in treated and latent cases of syphilis when the complement-fixation tests were negative, but in a broad and general manner it may be stated that the Meinicke, Sachs-Georgi, Vernes, direct and Kahn flocculation reactions are less sensitive than acceptable complement-fixation reactions conducted with acceptable reagents by individuals possessing the experience required and justified by the importance of the subject.

Specificity of the Precipitation Reactions.—As previously stated, normal serums possess the property of flocculating organic and inorganic colloids under certain conditions. By reason of the apparent technical simplicity of the flocculation tests it may be assumed that they are less subject to the biologic error of falsely positive reactions. But they are subject to this error in just the same manner as the Wassermann reaction and, indeed, in my opinion, to even greater degree because it is necessary to render the amount of flocculation as great as possible which tends to elicit non-specific reactions. Extracts employed as "antigens" in the flocculation tests are just as important from this standpoint as in the complement-fixation reaction, and particular care must be exercised with cholesterolized extracts.

Flocculation reactions also occur with the serums of individuals with frambesia (yaws) as would be expected. Whether or not reactions occur in the febrile stages of malaria, pneumonia and other acute infections, in diabetes, leprosy, and other diseases in which falsely positive Wassermann reactions are said to occur, cannot be stated. In my opinion we must revise our ideas concerning falsely positive Wassermann reactions because so many have been based upon technical errors, and I believe that the immunologic changes responsible for the complement-fixation and flocculation reactions occur only in spirochetic infections, notably syphilis and frambesia.

Practical Value of the Precipitation Reactions.—Summing up the subject it may be stated that the precipitation reactions are not as sensitive as the complement-fixation reactions conducted with a technically correct and acceptable technic; that they are more subject to misinterpretation and should not be relied upon *alone* for the serum diagnosis of syphilis. They may, however, be useful as controls on the complement-fixation test, and for this purpose the Meinicke (third modification), and more especially the Sachs-Georgi and Kahn reactions, are to be recommended.

CHAPTER XXV

COMPLEMENT FIXATION IN BACTERIAL INFECTIONS AND FOR THE DIFFERENTIATION OF PROTEINS

Specific Complement Fixation in Bacterial Diseases.—As has been stated elsewhere, the first complement-fixation tests were performed by Bordet with bacterial antigens and antisera (pest and typhoid). Following the application of the principles of complement fixation in the serum diagnosis of syphilis, it was but natural that the possibilities of this method as a general means of diagnosis soon became appreciated, and in a short time numerous infections were studied.

Probably in no disease has complement fixation proved so constant or so valuable a diagnostic procedure as in syphilis. In this condition the peculiar lipodophilic reagin is largely responsible for the marked fixation of complement, and from our present knowledge on the subject we learn that this phenomenon has practically no analogy in any other disease except frambesia.

With few exceptions bacterial antigens are likely to yield weaker and more inconstant reactions. This is due to the fact either that our antigen lacks a more available and specific antigenic principle, or that the amount of complement-fixing bodies is small and variable. For these reasons it becomes apparent that the preparation of antigen and sensitiveness of technic are highly important factors.

Complement Fixation in the Differentiation of Microparasites.—Several investigators have applied the technic of complement fixation in a study of the relationship and differentiation of closely related bacteria, spirochetes, trypanosomes, and other microparasites. These studies have usually been made by immunizing rabbits with a particular strain and using the immune serum in complement-fixation tests with antigens of the other microparasites under study. As a general rule complement fixation is most marked with homologous antigen and antiserum; relationship is studied according to whether or not complement fixation occurs with the other antigens. Besredka,¹ Foix and Mallein,² Swift and Thro³ have reported that immune amboceptors specific for different strains of streptococci can be demonstrated by means of the complement-fixation test. In my own work with five strains of streptococci,⁴ with the specific purpose of studying the relationship of the streptococcus commonly found associated with scarlet fever to the group of streptococci, I found that differentiation among these was possible when using high dilution of the immune sera, but in lower dilutions differentiation was not found by means of complement-fixation tests, the results indicating either that this scarlet fever streptococcus belonged to the common group of streptococci or that the complement-fixation test was a group reaction. In a similar study of the diphtheria group of bacilli I found that the pseudodiphtheria bacillus could not be differentiated from other members of the group by complement-fixation reactions⁵ indicating its relationship to the true diphtheria bacillus; similar studies made by Williams, Raiziss, and myself⁶ with the typhoid

¹ Ann. de l'Inst. Pasteur, 1904, 28, 363.

² Presse médicale, 1907, 15, 777.

³ Arch. Int. Med., 1911, 7, 24.

⁴ Arch. Int. Med., 1912, 9, 220.

⁵ Jour. Infect. Dis., 1912, 11, 44.

⁶ Jour. Infect. Dis., 1913, 13, 321.

colon group of bacilli, by Craig and Nickols¹ with several strains of spirochetes, by Cooke² with acid-fast bacilli, by Olmstead³ and Wollstein⁴ with meningococci, by Hooker⁵ with typhoid bacilli, and by Hitchens and Brown⁶ with streptococci indicate that the bacteriolytic amboceptors are highly specific and yield highly specific reactions with proper technic, and particularly with good and sensitive antigens, and under these conditions the complement-fixation test may be more delicate in differentiation than agglutination or other immunity reactions. As a practical procedure, however, the complement-fixation technic is too intricate and time consuming. *In this connection I desire to direct particular attention to the possibility of the sera of normal rabbits yielding positive complement-fixation reactions with various bacterial and lipoidal extracts as discussed on page 438; in conducting complement-fixation tests with rabbit- or dog-sera the serum of each animal should first be tested with the antigen and immunization conducted only in case the animal's serum is found to react negatively; subsequent complement-fixing properties of the serum may then be ascribed to the presence of specific amboceptors.*

TECHNIC OF BACTERIAL COMPLEMENT-FIXATION TESTS

Preparation of Bacterial Antigens.—Either the endotoxins or whole bacterial body may constitute the main portion of an antigen. Most recent efforts have aimed thoroughly to disorganize the bacterial cell in order to liberate the endotoxic substances that pass into solution and constitute the antigen. Experience has frequently shown, however, that the protein substances of the bacterial cell itself possess antigenic properties, and, accordingly, I have generally found that antigens composed of bacterial cells and the products of their activity are usually more satisfactory than those prepared of the endotoxic substances alone.

As a general rule, bacterial antigens should be polyvalent—i. e., made up of a number of different strains of the same micro-organism. Recent researches in bacteriology tend to show that different strains of the same micro-organism have particular and more or less individual pathogenic and sometimes biologic characteristics, and it is reasonable to assume that the antibody will likewise show individual properties and a special affinity for its particular antigen. When, therefore, one antigen is being used in complement-fixation work, the results are more likely to be satisfactory if a large number of different strains are included in the antigen, with the hope that at least one of them will show a particular affinity for the antibody in the patient's serum.

Bacterial antigens may be prepared in various ways.

Suspension in Broth or Saline Solution.—Cultures are grown in a suitable fluid medium, such as plain bouillon, for forty-eight hours, or upon a solid medium, and washed off with a suitable quantity of normal saline solution. The culture or emulsion is shaken for an hour or so to break up the clumps, and then heated to 60° C. for an hour. It is preserved by the addition of 0.25 per cent. of phenol or tricesol.

This constitutes the simplest bacterial antigen. It is composed of both bacterial cells and the products of bacterial activity, and frequently yields uniform and satisfactory results.

Schwartz and McNeil Method.—Cultures are grown on a suitable solid medium for from twenty-four to forty-eight hours. Growths are removed

¹ Jour. Exper. Med., 1912, 16, 336.

² Jour. Amer. Med. Assoc., 1917, lxxviii, 1504.

³ Jour. Immunology, 1916, 1, 307.

⁴ Jour. Exper. Med., 1914, 20, 201.

⁵ Jour. Immunology, 1916, 1, 1.

⁶ Personal communication.

by adding sufficient distilled water or normal saline solution to yield a milky suspension. The emulsion is heated to 56° C. in a water-bath for one hour, followed by one hour at 80° C., and shaken mechanically with glass beads for twenty-four hours, to facilitate disintegration. It is then filtered through paper pulp and a sterile Berkefeld filter with a *neutral reaction*, or thoroughly centrifugalized; the filtrate is then heated to 56° C. for one-half hour on each of three successive days to sterilize, and preserved with 0.25 per cent. phenol and used as antigen after being diluted and made isotonic with 1 part of 9 per cent. salt solution to 9 parts of the antigen.

This antigen is composed essentially of endotoxic substances, and is the one usually employed in the preparation of gonococcus antigen.

Antigens of Dried Bacteria.—Cultures are grown on a solid medium, washed off with normal saline solution, and the emulsion centrifuged thoroughly. The sediment is dried over sulphuric acid or calcium chlorid, and the dried material thoroughly ground with crystals of sodium chlorid. Sufficient distilled water is then added to render the solution isotonic, and so that it will contain about 0.5 gm. of dried material in each cubic centimeter. This emulsion is then shaken for twenty-four hours, filtered or centrifuged, the filtrate preserved with 0.5 per cent. of phenol, and used as antigen. Antigen may be prepared also as follows:

Cultures are grown on a solid medium and washed off with normal saline solution. Saline suspension is then precipitated with an equal quantity of absolute alcohol and centrifugalized. The sediment is dried *in vacuo* over sulphuric acid, weighed, and ground into a fine powder with sufficient crystals of sodium chlorid to make a 2 per cent. suspension of dried material in isotonic saline solution. This stock suspension is not filtered or centrifuged, but is further diluted with saline solution, and constitutes the antigen (method of Besredka, modified by Gay). The actual amounts of dry antigenic substance contained in 1 c.c. of various dilutions are as follows:

1 c.c. of 1 : 40 dilution	= 0.5 mg.
1 c.c. of 1 : 80 dilution	= 0.25 mg.
1 c.c. of 1 : 160 dilution	= 0.125 mg.
1 c.c. of 1 : 320 dilution	= 0.062 mg.
1 c.c. of 1 : 640 dilution	= 0.031 mg.
1 c.c. of 1 : 1280 dilution	= 1.0155 mg., etc.

Hitchens' and Hansen Method.—Young cultures of bacteria grown on solid media are suspended in distilled water (about 10 c.c. being used for each 20 square inches). To this suspension is added an equal volume of 95 per cent. ethyl alcohol followed immediately by centrifugalization. The precipitate is again suspended in alcohol and centrifuged; the precipitate is now suspended in ether, the original volume of the suspension being reduced to one-half each time. After removal of the supernatant ether the last traces of ether adhering to the precipitate are removed by vacuum. The bacterial mass is now dried over phosphorus pentoxid *in vacuo* for three days and stored in amounts of about 0.05 gm. in "H" tubes with phosphorus pentoxid *in vacuo*. Suspensions are prepared in saline solution as required.

Small's Method.—Twenty-four-hour growths of bacteria on solid medium are removed and dried in sterile Petri dishes at 53° to 56° C. The scaly mass is ground in a mortar while being moistened with chloroform followed by small additions of ether. The grinding is maintained until the last addition of ether has evaporated and a fine buff colored powder is obtained. This is now suspended in a mixture of equal parts of chloroform and ether and shaken for four to six hours. After settling the supernatant fluid is re-



FIG. 147.—ANTICOMPLEMENTARY TITRATION OF A GONOCOCCUS ANTIGEN.
The tube containing 0.6 c.c. shows slight inhibition of hemolysis, and this amount is the *anticomplementary unit*.

moved and the residue shaken up three or four times with ether. After decanting or filtering away the last ether the powder is dried in an incubator at 56° C.; likewise the residue on the filter-paper which is now recovered. The powder is now placed in sterile saline solution containing 0.5 per cent. phenol (25 c.c. for each 0.5 gm. of residue at the start), shaken for twelve to eighteen hours and used as antigen. This method was described by Small for the preparation of antigens of typhoid, paratyphoid and dysentery bacilli, pneumococci, and meningococci.

Special methods for the preparation of antigens of tubercle bacilli and other micro-organisms will be described in the special sections of this chapter devoted to these subjects.

TECHNIC OF COMPLEMENT-FIXATION TESTS

AUTHOR'S FIRST METHOD

This test is conducted in exactly the same manner as the author's modification of the Wassermann test employing multiple antigens described on pages 473 to 478. It is applicable for the gonococcus, tuberculosis, typhoid, or any other bacterial complement-fixation test.

Hemolytic System.—*Complement* is collected as described, diluted 1 : 20 and used in dose of 1 c.c. *Sheep corpuscles* are collected and washed as previously described, prepared in a 2.5 per cent. suspension, and used in dose of 1 c.c. *Antisheep hemolysin* is titrated as described on page 473 and used in dose of 2 units.

Titration of Bacterial Antigen.—After an antigen has been prepared it is standardized by determining the *anticomplementary unit*—i. e., the amount of antigen that just begins to show inhibition of hemolysis due to non-specific complement fixation. This unit is easily determined by adding increasing amounts of antigen to a series of test-tubes with a constant dose of complement in each. As a general rule, it is well to add to each tube a constant dose of *fresh normal* inactivated serum, e. g., as 0.1 to 0.2 c.c., when the anticomplementary action of serum alone is allowed for. I would emphasize the necessity of doing this in experimental work with rabbit-, dog-, or any other animal serum. After incubating for one hour, 2 units of hemolytic amboceptor and 1 c.c. of corpuscle suspension are added to each tube, and the tubes are reincubated for an hour or two and the reading made (Fig. 147). *In the main test one-quarter to one-half the anticomplementary unit may be used, as this amount is known to be free from any power of non-specific complement fixation.* The former dose is, of course, safer than the latter.

ANTICOMPLEMENTARY TITRATION OF A BACTERIAL ANTIGEN (FIG. 147)

TUBE.	ANTIGEN, 1 : 10, C.C.	COMPLEMENT, 1 : 20, C.C.	Saline solution in each tube to 2 c.c.; tubes are shaken and incubated for one hour at 37° C.	ANTISHEEP AMBO- CEPTOR, UNITS.	SHEEP'S CORPUSCLES (2.5 PER CENT.), C.C.	Tubes are shaken and incubated for one hour at 37° C.	RESULTS
1.....	0.2	1		2	1		Complete hemolysis.
2.....	0.4	1		2	1		Complete hemolysis.
3.....	0.6	1		2	1		Complete hemolysis.
4.....	0.8	1		2	1		Slight inhibition of hemolysis (unit).
5.....	1.0	1		2	1		Marked inhibition of hemolysis.
6.....	0	1		2	1		Hemolytic control. Complete hemolysis.

The titration may be completed by determining the *antigenic dose* of the antigen by titrating with a suitable and constant dose of specific immune serum. This titration is conducted by placing in a series of test-tubes increasing doses of antigen with a constant dose of heated immune serum (usually 0.1 c.c.) and a constant dose of complement. After an hour the proper dose of hemolytic amboceptor and corpuscles is added. The readings may be made an hour or two later, or after the tubes have been allowed to settle in a refrigerator. *That tube showing just complete inhibition of hemolysis contains the antigenic unit.* For the main test it is well to use double this amount, providing this dose is not more, and preferably less, than half the anticomplementary unit.

The antigenic titration is not always satisfactory, for when an artificial immune serum is used the concentration of antibodies may yield a much stronger reaction than one would expect in testing human serums. Further than this, the antibody content in antisera varies considerably, so that the antigenic unit fluctuates according to the particular serum used in making the titration. In general, therefore, it is sufficient to determine the anticomplementary unit and to use half or quarter this amount in performing the main test.

After antigens are prepared they may require further dilution with saline solution. This can be determined only by experience and as the result of a trial titration.

As watery extracts are prone to deteriorate, *it should be made a rule that the anticomplementary dose be determined each time before the main test is conducted.*

It has quite generally been proved that alcoholic extracts of bacteria do not yield satisfactory antigens, despite the advantage to be gained because of their stability.

The Test.—The serums should be fresh and clear, and heated to 56° C. for one-half hour. For each serum use four test-tubes (12 by 1 cm.) arranged in a row. Into each of the first three place the dose of antigen and increasing doses of serum—0.05 c.c., 0.1 c.c., 0.2 c.c.; the fourth tube is the serum control, and into this is placed the maximum dose of serum (0.2 c.c.), but no antigen; 1 c.c. of complement diluted 1 : 20 is added to each tube. The following *controls* are included:

1. A positive control with an immune serum or with the serum of a patient who reacted positively on a former occasion.

2. A negative control with the serum of a healthy person.

Both of these controls may be set up with but the maximum dose of serum (0.2 c.c.).

3. The serum control of each serum is conducted in the fourth tube of each series. At the completion of the test this tube should show complete hemolysis and thereby indicate that the serum was not anticomplementary.

4. The antigen control at this time includes the dose of antigen and complement.

5. The hemolytic system control at this time receives the dose of complement.

6. The corpuscle control receives 1 c.c. of the corpuscle suspension.

To each tube sufficient saline solution is added to bring the total volume up to about 2 c.c. The tubes are shaken and incubated for one hour at 37° C. in the thermostat or in a water-bath (not less than one hour), when 2 units of antishoop amboceptor and 1 c.c. of sheep corpuscle suspension are added to each tube except the corpuscle control. The tubes are gently shaken again and reincubated for one hour or longer, depending upon the



FIG. 148.—GONOCOCCUS COMPLEMENT-FIXATION REACTION.

Shows a + reaction with 0.05 c.c. serum; a ++ with 0.1 c.c., and a ++++ with 0.2 c.c., a strongly positive reaction.

hemolysis of the controls, after which the results are recorded. This secondary incubation may be omitted and the tubes placed in a refrigerator overnight and the results read the next morning. Under these conditions hemolysis occurs slowly, and, according to some workers in this field, the reaction becomes more delicate.

The following table is an example of a gonococcus fixation test with the serum of a case of gonorrheal arthritis (Fig. 148).

GONOCOCCUS COMPLEMENT-FIXATION TEST

PATIENT'S SERUM, C.C.	ANTIGEN, 1:10, C.C.	COMPLEMENT, 1:20, C.C.	Saline solution q. s. 2 c.c.; tubes are gently shaken and incubated for one hour at 37° C.	ANTISHEEP AMBO- CEPTOR, UNITS.	SHEEP COR- PUSCLES (2.5 PER CENT.), C.C.	RESULTS AFTER ONE AND A HALF HOURS' INCUBA- TION.
0.05	0.2	1		2	1	Slight inhibition of hemolysis.
0.1	0.2	1		2	1	Marked inhibition of hemolysis.
0.2	0.2	1		2	1	Complete inhibition of hemolysis.
0.2	0	1		2	1	Serum control: hemolysis.
Positive serum, 0.2	0.2	1		2	1	Complete inhibition of hemolysis.
0.2	0	1		2	1	Serum control: hemolysis.
Negative serum, 0.2	0.2	1		2	1	Hemolysis.
0.2	0	1		2	1	Hemolysis.
0	0.2	1		2	1	Antigen control: hemolysis.
0	0	1		2	1	Hemolytic control: hemolysis.
0	0	0		0	1	Corpuscle control: no hemolysis.

In reading the results the controls are first examined and should show complete hemolysis; the test is reported as negative if all tubes are hemolyzed, weakly positive if the largest dose only of serum (0.2 c.c.) shows inhibition of hemolysis, moderately positive if the 0.1 and 0.2 c.c. doses of serum show inhibition of hemolysis, and strongly positive if the 0.05, 0.1, and 0.2 c.c. doses of serum react positively.

The test may be conducted with but one dose of serum, namely, 0.2 c.c. with the antigen and 0.2 or 0.3 c.c. in the serum control tube. When conducting the Wassermann and gonococcus tests with the same serum an extra tube is simply added to the Wassermann series with three antigens as described on pages 473 to 470, making a five tube test for conducting both Wassermann and gonococcus tests at the same time. In this case the readings are made after the usual +, ++, +++, and +++++ method (see page 472).

AUTHOR'S METHOD BASED UPON STUDIES IN THE STANDARDIZATION OF TECHNIC

The test is conducted in exactly the same manner as the syphilis reaction described on page 478. Human serum seldom contains as much complement-fixing antibody in gonorrhea, tuberculosis, typhoid fever, and other bacterial infections as may be present in syphilis, and for this reason the amounts of serum employed should not vary too greatly. The qualitative test is conducted with two tubes in exactly the same manner as described.

The test has yielded much more sensitive reactions than the method described above; one important reason for this is because the primary incubation is eighteen hours at 6° to 8° C. instead of one hour in a water-bath.

The antigen must be titrated in exactly the same manner as described for the titration of syphilis antigens. As a general rule the anticomplementary titration only is conducted. If it is desired to conduct this titration just before the main tests are set up, the primary incubation may be one hour in a water-bath at 37° C., but in this case one-quarter the anticomplementary unit of antigen should be employed. If the antigen is titrated with the period of cold primary incubation it may be used in the main tests in one-third of the anticomplementary unit.

COMPLEMENT FIXATION IN GONOCOCCUS INFECTIONS

This was one of the first infections to be studied by means of the complement-fixation technic, but the results secured were not generally satisfactory until it was shown that the antigen must be polyvalent.

Historic.—In 1906 Muller and Oppenheim¹ applied the complement-fixation test to the diagnosis of gonorrheal arthritis, using a culture of the gonococcus as antigen. To these observers, therefore, belongs the credit of being the first to record a complement-fixation test in a gonococcus infection. A little later in the same year Carl Bruch² applied the reaction to 3 cases of gonorrhea, using the serum of immunized rabbits, and reported favorable results. In 1907 Meakins³ reported having secured positive reactions in 3 cases of gonorrheal arthritis, which was the first report in America published on this subject. Th. Vannod⁴ studied the specificity of the reaction with the serums of rabbits immunized with gonococcus protein and one of a meningococcus, and reported that the meningococcus immune serum did not show complement fixation with gonococcus antigen, and, vice versa, that gonococcus amboceptor was not bound by meningococcus antigen. Wollstein⁵ (1907), in a study of the biologic relationship of the gonococcus and meningococcus, reported findings differing from those of Vannod. The former observer found that bacteriolytic amboceptors in the serums of rabbits immunized with these cultures were closely related and yielded fixation of complement with either antigen. Teaque and Torrey⁶ in 1907 issued a very important communication showing that the differences in results of previous investigators were probably due in part to the use of single strains of the organisms in the preparation of antigens and immune serums. They emphasized the fact that the gonococcus belongs to a heterogeneous family, and that in attempting to formulate a diagnosis of gonorrheal infection by the complement-fixation method the extracts of several different strains should be used. Naz Vannod and later Watabiki⁷ found that the gonococcus and meningococcus antibodies were quite specific for their homologous antigens in complement-fixation reactions.

Particular attention was drawn to the gonococcus complement-fixation test by the work of Schwartz and McNeal.⁸ These investigators emphasized the necessity of using polyvalent antigens, and their encouraging reports have stimulated renewed interest in this subject. They found that if the infection is confined to the anterior urethra, a positive reaction is not obtained; that a strong reaction is not to be expected before the fourth week of the infection, and then only in acute cases with complications. They regard a positive reaction as indicating the presence or recent activity in the body of a focus of living gonococci, although a negative reaction does not exclude gonococcus infection. The test, therefore, has a more positive than a negative value. With Flexner's antimeningococcus serum positive reactions resulted with their gonococcus antigen; with serums from cases of cerebrospinal meningitis (meningococci) the results were negative.

In the succeeding years numerous investigators, including Swinburne, Gradwohl, O'Neil, Gardner and Clowes, Thomas and Ivy, Kolmer and Brown, Smith and Wilson, Dixon, Kilduffe, Lailey, and Cruickshank have reported favorably upon the practical value of the gonococcus complement-fixation test, particularly as an aid in determining whether or not a patient is cured of the infection.

¹ Wien. klin. Wchn., 1906, 19, 894.

² Deutsch. med. Wchn., 1906, 70, 36.

³ Johns Hopkins Medical Bull., 1907, 18, 255.

⁴ Ztschr. f. Bakter., 1907, 44, 10.

⁵ Jour. Exp. Med., 1907, 9, 588.

⁶ Jour. Med. Research, 1907, 17, 223.

⁷ Jour. Infect. Dis., 1910, 7, 159.

⁸ Amer. Jour. Med. Sci., May, 1911; *ibid.*, September, 1912; *ibid.*, December, 1912.

Antigen.—This constitutes the most important ingredient of the test. As Teague and Torrey¹ and Schwartz and McNeal² have emphasized, the antigen should be prepared of many different strains of gonococci. The difficulty of isolating this organism and the constant care required in subculturing and keeping a large number of strains alive render it practically impossible for many persons to prepare a gonococcus antigen. Therefore until simpler methods are devised, this antigen is best prepared in large central laboratories, where the cultures are handled and preserved by specially trained persons.

Torrey and Buckell,³ however, have recently described a single culture-medium for the gonococcus whereby strains are readily carried along so that antigen may be freshly prepared as required. As a result of very extensive immunologic studies with gonococci they have been able to select two strains for this purpose, each covering a large number of substrains.

The gonococci are well grown on a salt-free veal agar, neutral in reaction to phenolphthalein, and to which a few drops of sterile hydrocele fluid may be added. After culturing for from twenty-four to forty-eight hours the growths are washed off with distilled water, and the emulsion is heated in a water-bath for two hours at 56° C. It is then heated at 80° C. for one hour, filtered through paper pulp or centrifugalized, and passed through a Berkefeld filter which is reserved for this purpose alone and is neutral in reaction. A small amount of preservative, as, *e. g.*, 0.1 c.c. of a 1:100 dilution of phenol to each cubic centimeter of antigen, may be added. The antigen is then well preserved in small amounts in ampules that are sealed and heated to 56° C. for half an hour on three successive days. Just before being used the antigen is made isotonic by adding 1 part of a 10 per cent. salt solution to 9 parts of antigen. I preserve the antigen in ampules containing 1 c.c., and after removing the antigen from the ampule to a large test-tube, add 1 c.c. of 10 per cent. salt solution, and dilute the whole 1:10 with the addition of 8 c.c. of normal salt solution, after which the anti-complementary titration is made.

In this method of preparing the endotoxins constitute the main antigenic principle. Brown and myself,⁴ after an experimental study of the various antigens, found that a simple suspension of gonococci in salt solution yielded slightly better results. The various strains are grown for from forty-eight to seventy-two hours, and are then washed off with sterile saline solution, observing particular care not to include portions of the culture-medium. The suspension is then shaken to break up clumps, and heated to 56° C. for one hour. A small amount of preservative is now added, and the antigen stored in 1 c.c. ampules. Before using it is diluted 1:10 or 1:20, and titrated for the anticomplementary dose.

Alcoholic extracts of gonococci have very little practical value, as alcohol is not satisfactory for extracting the antigenic principles of bacteria. Warden,⁵ however, has reported good results with a new lipid antigen.

Specificity of the Gonococcus Complement-fixation Test.—Viewed from a practical standpoint, the reaction is highly specific. While complement-fixation experiments with antigens of gonococci and meningococci and their respective immune serums have demonstrated a biologic relationship between these micro-organisms, yet practically with human serums an antigen of pure cultures of gonococci will fix complement only with the gonococcus antibody (amboceptor). In this technic a specific antigen is employed, and

¹ Jour. Med. Research, 1907, 17, 223.

² Amer. Jour. Med. Sci., 1911, 141, 693.

³ Jour. Immunology, 1922, 7, 305.

⁴ Jour. Infect. Dis., 1914, 15, 6.

⁵ Jour. Amer. Med. Assoc., 1915, lxx, 2080; Jour. Lab. and Clin. Med., 1916, i, 333.

it is, therefore, a true application of the Bordet-Gengou reaction of complement fixation by specific antigen and specific antibody (amboceptor). Obtained under proper technical conditions, a positive reaction is invariably reliable, and indicates the presence of a focus of living gonococci.

Practical Value of the Gonococcus Complement-fixation Test.—From our present knowledge of this reaction it may be stated:

1. That the difficulty of isolating and preserving a sufficient number of cultures of true gonococci in order to prepare a satisfactory polyvalent antigen constitutes a weighty drawback to the practical use of the test.

2. Because the gonococcus antibody, unless complicated by wide-spread gonococcal metastases, is produced in small amount in the majority of cases, the degree of complement fixation is usually much less than that which occurs in the syphilis reaction, and accordingly the reactions are usually weaker and often indefinite. *A negative reaction does not exclude gonorrhea.*

3. The reaction is seldom positive during the first four to six weeks of an acute anterior or posterior urethritis, in the absence of complications. In acute exacerbations of a chronic urethritis the reaction is positive in about 80 per cent. of cases. In ordinary chronic urethritis with mild infection of the prostate gland the reaction is positive in from 30 to 40 per cent. of cases. In chronic urethritis complicated by marked involvement of the prostate gland and epididymitis the reactions are frequently positive, occurring in from 50 to over 80 per cent. of cases.

The test possesses considerable value in determining the fitness of an applicant for a marriage license, and will, no doubt, be employed for this purpose quite extensively, as a positive reaction is now generally regarded as indicating the presence of a focus of active gonococcal infection. A negative reaction, however, does not exclude the possibility of latent infection in the urethra and accessory organs.

4. During the course of an acute or a subacute urethritis the occurrence of an acute complication, such as prostatitis, epididymitis, etc., is likely to result in a positive fixation test.

5. In gonorrheal iritis a positive reaction occurs in over 80 per cent. of cases.

6. A positive reaction may persist for several weeks after the patient is clinically cured. Torrey¹ has shown experimentally that the antibody persists in the blood of rabbits artificially immunized for from ten days to six or seven weeks. Usually, under proper treatment, the reaction in ordinary cases of urethritis disappears in from two to three weeks; if, however, a positive reaction persists, a focus of infection is probably present, and the patient should be kept under further observation and the treatment persisted in.

7. In women the reaction is seldom positive until the infection has reached the cervical canal. In the case of little children, however, we have known positive reactions to occur in acute and chronic vulvovaginitis, indicating either that the disease is more severe in children, with more antibody formation, or that it may reach the cervical canal.

The reaction is positive in about 60 per cent. of cases of pyosalpingitis, and the test may prove of value in making the differentiation of inflammatory lesions from certain cystic and neoplastic conditions, and in establishing the gonorrheal basis of many of these infections.

In a recent thorough study by Torrey, Wilson and Buckell² of the comparative value of smears, cultures, and complement-fixation tests in the diagnosis of chronic gonorrhea of women the following results were observed in 56 cases as taken from their report:

¹ Jour. Med. Research, 1910, i, 95.

² Jour. Infect. Dis., 1922, 31, 148.

COMPARISON OF SMEAR, CULTURE, AND COMPLEMENT FIXATION IN VARIOUS STAGES OF GONORRHEA IN WOMEN

DIAGNOSIS.	NUMBER OF CASES.	PERCENTAGE POSITIVE DIAGNOSES.		
		Smear.	Culture.	Complement Fixation.
Acute gonorrhea.	1	Not stated.	Positive.	Positive.
Subacute gonorrhea.	8	50 per cent.	50 per cent.	50.0 per cent.
Chronic gonorrhea.	33	12 "	20 "	69.5 "
Doubtful gonorrhea.	14	14 "	28 "	71.0 "

The authors concluded that the complement-fixation test was of particular value in the diagnosis of chronic gonorrhea, although smear and cultural methods may be better criteria of cure.

8. Cases of gonorrheal arthritis yield from 80 to 100 per cent. of positive reactions, and the complement-fixation test has considerable value in establishing the diagnosis of these infections.

9. *The administration of gonococcus vaccine and antigonococcus serum is likely to be followed by positive reactions.* Just how long the antibodies may persist in the blood after a clinical cure has been effected it is difficult to state; at least from six to twelve weeks' time should be given for them to disappear.

10. In medicolegal cases the courts may not accept the usual evidence offered by a bacteriologic diagnosis based upon stained smears of a secretion, and cultures are frequently differentiated from other Gram-negative diplococci only with difficulty. Conducted with the proper technic the gonococcus fixation test is highly specific and much less difficult to perform.

11. Finally, it must be emphasized that the reaction has a far more positive than negative value. The reaction is highly specific, but there is a limit to its delicacy, so that a negative reaction in urethritis does not exclude the possibility of gonococcal infection.

COMPLEMENT FIXATION IN GLANDERS

The complement-fixation test is used extensively by veterinarians in making a laboratory diagnosis of glanders. The test has been found very reliable, and is usually more delicate than the agglutination test and the Strauss guinea-pig test. It has also been used successfully in the diagnosis of human glanders.

Preparation and Standardization of Antigen.—The antigen should be polyvalent, and composed of at least several different strains. Cultures of *Bacillus mallei* are grown on slants of glycerin agar (1.6 per cent. acid) for from forty-eight to seventy-two hours. The growths are then removed, and sufficient distilled water added to give a milky suspension. This suspension is sterilized by heating the tubes to 60 C.° for two hours. They are then shaken mechanically with glass beads for a few hours on two successive days. Enough sodium chlorid is added to make the solution isotonic, and the whole is preserved with 0.25 per cent. phenol and stored in a dark, cold place, where it will keep for many months. Antigen may also be prepared in the same manner as gonococcus antigen as described on page 545 and employed by Wade.¹

¹ Jour. Infect. Dis., 1913, 12, 7.

A simpler antigen is prepared by growing the bacillus in glycerin bouillon for seventy-two hours, sterilizing by heating to 60° C. for two hours, and preserving with the addition of 0.25 per cent. of phenol.

COMPLEMENT FIXATION IN CONTAGIOUS ABORTION

It is now generally conceded among veterinarians that the *Bacillus abortus* of Bang is the specific cause of contagious abortion of cows.

Evidence is gradually accumulating to show that an organism belonging to the paratyphoid group is frequently the cause of a similar condition among mares (Kilbourne and Smith,¹ Liguierer,² Liguierer and Zabala, Good,³ Van Neelsbergen,⁴ de Jong,⁵ Meyer and Boerner⁶). Meyer and Boerner, who have studied this bacillus with particular care, classify it with the paratyphoid enteritidis group (*Bacillus aborti equi*).

Veterinarians are generally agreed that in contagious abortion of cows the complement-fixation test is highly specific; frequently of considerable value in establishing a diagnosis (Meyer and Hardenburgh and others).

Meyer and Boerner have found fixation of complement to occur in contagious abortion of mares with an antigen of *Bacillus abortus equi*, and recommend the test as diagnostic aid in this infection.

Preparation of Antigen.—The antigen of *Bacillus abortus* (Bang) for use in the complement-fixation test in contagious abortion of cows is prepared by cultivating a number of strains of the bacillus, which have been trained to grow aërobically, upon slants of glycerin agar for seventy-two hours. The growths are then washed off with sufficient normal saline solution containing 2 per cent. phenol to yield a cloudy emulsion. Shake briskly in order to break up the clumps of bacilli, and filter through paper. Place in a refrigerator for several days to complete the sterilization, and titrate the anticomplementary dose each time before the main test is conducted.

The antigen may also be prepared by cultivating a number of strains in glycerin-serum bouillon for five or six weeks. Centrifuge thoroughly and wash the bacilli once or twice with normal saline solution to remove all traces of serum. Dilute the washed bacilli with sufficient normal saline solution to give an emulsion equal in density to a twenty-four-hour bouillon culture of *Bacillus coli*, and add 0.4 per cent. of phenol as a preservative.

The antigen of *Bacillus abortus equi* for making the complement-fixation diagnosis of contagious abortion of mares is prepared of eighteen- to twenty-hour-old glycerin bouillon cultures, with an addition of 0.5 per cent. of phenol. These antigens are less anticomplementary than shake extracts, and keep their titer unaltered for many weeks (Meyer and Boerner). They may also be used for making the macroscopic agglutination test. These antigens may also be prepared after the method used in the preparation of gonococcus antigen (page 545).

COMPLEMENT FIXATION IN DOURINE

Dourine, or horse syphilis, is a specific infectious disease of the horse and ass, transmitted from animal to animal by the act of copulation, and caused by the *Trypanosoma equiperdum*. It is characterized by an irregular

¹ United States Department of Agriculture, Bulletin No. 3, 1893, 49, 53.

² Rec. Med. vétérinaire, lxxxii, 1905.

³ Kentucky Agriculture Exper. Station Bulletin No. 165, 1912.

⁴ Tijdschrift, v. Veeartsenijk., xxiv, 1912.

⁵ Archiv. f. Wissenschaftl. u. prak. Tierheilkunde, xxv, 1900.

⁶ Jour. Med. Research, 1913, xxix, No. 2, 325.

incubation period, the localization of the early symptoms to the genital organs, and, finally, by complete paralysis of the posterior extremities, a fatal termination ensuing in from six months to two years.

The disease is especially prevalent among horses in the northwestern states, and may occur in such various and atypical forms as to render clinical diagnosis difficult.

Complement-fixation methods of diagnosis have been tried by Pavlosvici, Winkler and Wyschelersky, Moller, Watson, Brown, and in a large series of cases with good results by Moller, Eichhorn, and Buck.¹ These last-named investigators examined 8657 specimens of blood from horses in Montana and North and South Dakota, and of these, 1076 yielded positive reactions.

In most of these experiments the results were corroborated by clinical and pathologic findings, and the investigators conclude that the complement-fixation test is of great value, especially in countries where only one of these protozoan diseases exists.

Preparation of Antigen.—This is the most difficult part of the technic, because the trypanosome is not readily grown on artificial culture-media. Watery, alcoholic, and acetone extracts of various organs of horses dead of the disease do not yield satisfactory antigens. Since the reaction is a group reaction, and dourine is the only trypanosome infection in this country, Moller, Eichhorn, and Buck selected the surra organism for the preparation of antigen. After infecting a dog and at the height of infection withdrawing 200 c.c. of blood into potassium citrate and hemolyzing with 0.5 gm. of saponin, the trypanosomes were secured after thorough centrifugalization and washed three times. After the last washing the trypanosomes were emulsified in 50 c.c. of salt solution and preserved with phenol. This antigen yielded highly satisfactory results, but the difficulty of preparing it, and the small quantity secured, made it necessary that another method be used.

An extract of the spleen of a rat just dead of surra was found to yield a satisfactory antigen. The extract does not keep well, and must be prepared freshly every few days and carefully standardized. Gray or white rats are infected with surra by injecting 0.2 c.c. of blood from a seed rat or rabbit with this disease. If a large number of tests are to be made, the rats should be so infected that one or two are available each day for the preparation of the antigen.

The spleen from a rat with a small amount of salt solution added is ground in a mortar until a pulpy mass results. More of the salt solution is added from time to time, and the suspension thus obtained is filtered twice through a double layer of gauze and diluted with salt solution to 40 c.c. I have secured better preparations by using the *liver* instead of the spleen; such antigens show the presence of more trypanosomes.

The anticomplementary and antigenic units are then determined, and the extract used in double the antigenic unit, providing that this amount is not more than half the anticomplementary unit. If a positive serum from an infected horse is not available, the anticomplementary unit may be determined and half this amount used in conducting the main test.

COMPLEMENT FIXATION IN TYPHOID AND PARATYPHOID FEVER

Typhoid fever was one of the original diseases in which Bordet and Gengou first demonstrated the occurrence of complement fixation. Widal and Lesourd attempted to make practical application of this method in the diagnosis of the disease, but their results were indifferent, and since

¹ Amer. Jour. Veter. Med., 1913, viii, 581.

then numerous writers have expressed various opinions as to the value of the test. Garbat¹ has secured uniform and reliable reactions with a polyvalent antigen, and emphasizes the importance of this factor. He² has found that practically all individuals with typhoid fever develop complement-fixing antibodies sooner or later during the course of the disease and that the test possesses diagnostic value. He also observed that people inoculated with the triple typhoid and para typhoid vaccine gave positive reactions only occasionally and for a very short time. For this reason Garbat believes that the test becomes of special value as a differential diagnostic aid in patients who are suspected of having typhoid fever, and give a positive Widal reaction due to previous vaccination, but in whom no typhoid bacilli can be found in the blood or excretions. Felke³ has arrived at the same conclusion, and Hage and Korff-Petersen⁴ found that the serum of vaccinated individuals may yield positive reactions for at least two months.

In my experience the typhoid complement-fixation test with polyvalent antigens has proved a valuable diagnostic aid for typhoid fever. *Not infrequently the tests have yielded positive reactions earlier than the Widal test*, employing the usual 1:40 and 1:80 dilutions of serum. Likewise the serums of rabbits immunized with living and dead typhoid bacilli have usually shown that complement-fixing antibody is produced very promptly and usually several days before the agglutinins are detectable. Hadjopoulos⁵ has also found that complement fixation is one of the earliest and most constant reactions in typhoid fever.

Vaccination with the usual three doses of triple vaccine results in the production of complement-fixing antibody in practically all individuals. As found by Garbat, these antibodies usually disappear after a month or two, but Berge and myself⁶ have observed positive reactions for as long as four months, and in one instance, for about two years. In the serum diagnosis of typhoid fever in an individual previously vaccinated, I believe it is advisable to conduct the tests with varying amounts of serum at intervals of two or three days. If typhoid infection is present a rise in complement-fixing antibody is observed; if typhoid is not present the complement-fixing titer of the serum remains fairly constant in much the same manner as the agglutinin curve determined by the Dreyer method.

The complement-fixation test will probably prove of value in the diagnosis of *paratyphoid fever*, employing antigens of paratyphoid bacilli. I have had no actual experience with the test for this purpose employing human serums, but with experimental infections in rabbits the complement-fixation reaction has proved more sensitive than the agglutination reaction for detecting and differentiating paratyphoid infections. It is necessary, of course, to use varying amounts of serum when conducting the tests in order to avoid group reactions.

Preparation of Antigen.—The antigen is prepared of numerous strains of typhoid bacilli—the more, the better. In Garbat's method cultures are grown on slants of agar for twenty-four hours, washed off with small quantities of sterile distilled water (about 1 c.c. to an agar slant), heated to 60° C. for twenty-four hours, shaken mechanically for twenty-four hours, and thor-

¹ Amer. Jour. Med. Sci., 1914, 148, 84; Jour. Immunology, 1916, 1, 391.

² Monograph No. 16 of the Rockefeller Institute, May 10, 1922, 82.

³ Münch. med. Wchn., 1915, 52, 578.

⁴ Deutsch. med. Wchn., 1915, 41, 1328.

⁵ Jour. Infect. Dis., 1922, 31, 226.

⁶ Jour. Immunology, 1916, 1, 409.

oughly centrifugalized for four to eight hours until the supernatant fluid is absolutely clear. The filtrate is preserved with 0.25 per cent. phenol and used as antigen. The antigen is titrated for anticomplementary activity and used in one-half this amount.

I have secured good results by removing the growths with small amounts of normal salt solution, and placing them in a shaking flask, and shaking for one hour to break up the clumps. After heating to 60° C. for one hour, 1 per cent. glycerin and 0.25 phenol are added as preservatives, and the mixture stored away in ampules containing 1 c.c. each. The emulsion should be slightly milky in appearance. A comparative study of nine methods for preparing typhoid antigens by Motsumoto,¹ in my laboratory, showed that these simple suspensions utilizing the broken up bodies of the bacilli were more satisfactory than filtrates or centrifugates of autolyzed bacilli.

COMPLEMENT FIXATION IN TUBERCULOSIS

It was the original studies in complement fixation in tuberculosis made by Wassermann and Bruch that later induced these workers, in co-operation with Neisser, to apply the method to the diagnosis of syphilis.

The first application of complement-fixation in tuberculosis was made by Widal and LeSourd² in 1901. They obtained deviation of complement in certain cases of tuberculosis, using as antigen homogeneous emulsions of tubercle bacilli of the Arloing-Courmont strain. In 1903 Bordet and Gengou³ demonstrated the presence of antibody capable of uniting with tubercle bacilli and fixing complement in the sera of tuberculous animals. Wassermann and Bruch⁴ in 1906 demonstrated the presence of an antibody to tuberculin in patients treated with tuberculin, but they examined only 13 cases of pulmonary tuberculosis. Caulfield⁵ in 1911 examined 104 cases of pulmonary tuberculosis with bacillary emulsion as antigen and obtained 33 per cent. Turban I cases, 70 per cent. Turban II, and 62 per cent. Turban III positive results. Laird⁶ (1912), out of 84 tests in 34 cases, obtained 24 positives in 4 cases, using watery emulsion of tubercle bacilli (which he does not describe); his results were inconclusive. Hammer,⁷ using O. T. and extracted tuberculous nodules, obtained 97 per cent. positive results in 46 tuberculous cases. Calmette and Massol,⁸ using preparations made from tubercle bacilli by extracting with water and peptone, obtained in 134 cases 92.5 per cent. fixation. Fraser⁹ (1913), testing a large variety of antigens, found that living bacilli gave no fixation in 96.6 per cent. of normal individuals, but gave positive reactions in 42.3 per cent. of tuberculous individuals. She states that the most reliable antigen is prepared from living human bacilli, and that diagnostically the complement-fixation test with living bacilli is of more value from the standpoint of positive results than any other reaction discovered to date. She believes the absence of antibodies accounts for the low percentage of results obtained. Dudgeon, Meck, and Weir¹⁰ also tested a large number of antigens, and in 102 untreated cases obtained 86 positive results, while all cases which had been treated with tuberculin gave positive results. Products of the bacilli themselves were found to be the most satisfactory as antigen. With an alcoholic

¹ Jour. Immunology, 1920, 5, 111.

² Cited by Shennan and Miller, Edinburgh Med. Jour., 1913, 10, 81.

³ Compt. rend. Acad. de Sci., 1903, 137, 351.

⁴ Deutsch. med. Wchn., 1906, 32, 449.

⁵ Jour. Med. Research, 1911, 24, 122.

⁶ Jour. Med. Research, 1912, 27, 163.

⁷ Münch. med. Wchn., 1912, 59, 1750.

⁸ Compt. rend. Soc. de biol., 1912, 73, 120.

⁹ Ztschr. f. Immunitätsf., 1913, 20, 291.

¹⁰ The Lancet, 1913, 184, 19.

antigen¹ prepared from tubercle bacilli they obtained from a total of 234 cases, 209 (89.3 per cent.) positives, 194 of these on first examination, 11 (of the 15 negative) on second examination, and 4 more on third examination. Besredka² (1913) prepared an antigen by growing tubercle bacilli on egg broth, heating it, and filtering. With this antigen Bronfenbrenner³ (1914) obtained a very high percentage of positive results, 93.8 per cent. in active cases, and 55.5 per cent. in convalescents, while suspected cases gave 75 per cent. and syphilitic sera 24 per cent. positive reactions. Inman⁴ and Kuss, Leredde and Rubenstein⁵ found this antigen non-specific. McIntosh, Fildes, and Radcliffe⁶ (1914) also justly criticized Besredka's antigen, and concluded, after testing a large number of antigens, that the living bacillary emulsion was best, yielding 76.7 per cent. positive results in 43 definite cases of phthisis, 80.7 per cent. in surgical tuberculosis, and 37.5 per cent. in glandular tuberculosis. Of 87 normal individuals, only 3 gave positive reactions (2 of these were lepers and 1 had Addison's disease). Negative reactions were obtained in 18 syphilitic patients. They look upon a positive reaction as indicative of active tuberculosis. Stimson⁷ (1915), who gives a fairly exhaustive table of the recent literature, reports a small number of cases in which a variety of antigens were used, but his results were inconclusive. Craig⁸ (1915) reports the results of examination of 166 cases of pulmonary tuberculosis in which he employed as antigen an alcoholic extract of several strains of human tubercle bacilli which had been grown on a liquid medium of alkaline broth containing egg; 96.2 per cent. positive results were obtained in active cases and 66.1 per cent. positive in inactive cases. One hundred and fifty cases of syphilis gave only 2 positive reactions, and these, on further examination, revealed lesions in the lungs. One hundred other diseases examined all gave negative results. In a later report Craig, using his same antigen of a filtrate of an alcoholic extract of several strains of human tubercle bacillus plus the culture-medium in which the bacilli had been grown, has reported further favorable results in complement fixation in tuberculosis. Miller and Zinsser⁹ have described a simple antigen prepared by grinding living or dead tubercle bacilli with dry table salt, and then adding distilled water up to isotonicity. With this antigen they have reported excellent results; Miller¹⁰ found the reaction practically always positive in active tuberculosis with negative reactions in non-tuberculous syphilitic and normal persons. In my own studies in co-operation with Montgomery, antigen prepared after Miller's method, yielded positive reactions only with the sera of tuberculous persons, but the percentage of positive reactions was much less than reported. Positive reactions were also occasionally observed with the serum of actively syphilitic individuals presenting no clinical evidence of tuberculosis. Corber,¹¹ employing an autolysate of tubercle bacilli as antigen, found 30 per cent. positive reactions with clinically definite cases of tuberculosis both active and inactive. Active cases gave a higher percentage of positive results than inactive cases. Eichhorn and Blumberg,¹² using Besredka's antigen and the sera of tuberculous persons and the lower animals, found the complement-fixation test less reliable than the subcutaneous tuberculin test in the diagnosis of tuberculosis

¹ Jour. Hyg., 1914, 14, 52, 72.

⁴ Compt. rend. Soc. de biol., 1914, 76, 251.

² Compt. rend. Acad. d. sc., 1913, 156, 1633.

⁵ Compt. rend. Soc. de biol., 1914, 76, 244.

³ Arch. Int. Med., 1914, 14, 786.

⁶ The Lancet, 1914, 185, 485.

⁷ Bull. Hyg. Lab. U. S. P. H. and M. H. S., 1915, No. 101, 7.

⁸ Amer. Jour. Med. Sci., 1915, 150, 781; Jour. Amer. Med. Assoc., 1917, lxxviii, 773.

⁹ Proc. Soc. Exper. Biol. and Med., 1916, xiii, 134.

¹⁰ Jour. Amer. Med. Assoc., 1916, lxxvii, 1519.

¹¹ Jour. Infect. Dis., 1916, 19, 315.

¹² Jour. Agr. Research, 1917, viii, 1.

in cattle and not practical for general diagnostic purposes. Lucke¹ found that the wax of tubercle bacilli possessed no antigenic properties; Wood, Bushnell, and Maddux² have observed a large percentage of apparently specific reactions with the partial antigens of Deyke and Much.³ Bronfenbrenner⁴ has reported very favorably upon the diagnostic value of the test employing Besredka's antigen; also von Wedel⁵ employing Wilson's antigen. Moon,⁶ McCaskey,⁷ and others have made favorable reports.

Preparation of Antigen.—It is apparent, therefore, that the question of proper antigen is of paramount importance in complement fixation in tuberculosis. The main objections to the bacillary emulsion are the small difference between the antigenic and anticomplementary units, the turbidity, and fairly high percentage of non-specific reactions. Eichhorn and Blumberg have given the following directions for the preparation of a modified Besredka antigen:

Twenty c.c. each of the white and the yolk of an egg are thoroughly beaten in an automatic egg-beater, and to the whipped material a solution of Liebig's meat extract (3 : 1000) in distilled water is gradually added while the mixture is continuously beaten. A sufficient meat-extract infusion is used to make up the whole to 1000 c.c. The emulsion is strained through cotton and heated to the boiling-point, then strained again, and after the addition of 0.5 per cent. of sodium chlorid is carefully neutralized, heated again and strained, and neutralized if necessary. To the neutral medium sufficient normal sodium hydroxid solution is added to make the medium of 0.2 alkalinity. This medium, to which neither peptone nor glycerin is added, is then autoclaved at 115° C. for twenty minutes, and after cooling is kept at 37° C. for forty-eight hours for observation as to sterility.

Miller-Zinsser Antigen.—Young cultures are removed from a solid medium with saline solution autoclaved and centrifuged. To each 0.020 gm. of bacterial residue in a centrifuge tube add 0.090 gm. of sodium chlorid and stir with a glass rod for one hour; add 10 c.c. of water.⁸

Wilson Antigen.—Broth cultures (tubercle bacilli grown for three weeks in glycerin-broth) are sterilized by heating in an Arnold sterilizer for one hour followed by filtering through paper. To each volume of residue is added ten volumes of alcohol, shaken by hand and extracted in a refrigerator for two weeks. This emulsion is then filtered through paper and the filtrate discarded, the residue being washed in absolute alcohol, secured by centrifugalization and washed twice with ether. The residue is now dried in the centrifuge tube overnight in an incubator and each gram emulsified in 200 c.c. of saline solution, heated for one hour at 80° C. and kept in a refrigerator as stock antigen.⁹

Petroff's Antigen.—Washed bacteria are dried and thoroughly pulverized. One gram of this residue is triturated in a mortar with 100 c.c. of 25 per cent. glycerin in water and slowly boiled in a flask having a reflex condenser. The material is now centrifuged or set aside for a few hours and the supernatant fluid used as antigen. This is one of the methods described by Petroff¹⁰ for the preparation of an antigen.

¹ Jour. Immunology, 1916, 1, 457.

² Jour. Immunology, 1917, 2, 301.

³ Med. Klinik., 1908, 4, 1540; Münch. med. Wehnschr., 1909, 56, 1895; *ibid.*, 1909, 56, 1825; Beitr. z. klin. Tuberk., 1910, 15, 277; *ibid.*, 1911, 20, 343.

⁴ Jour. Lab. and Clin. Med., 1917, 3, 51.

⁵ Jour. Immunology, 1920, 5, 159 (also gives a good review of the literature).

⁶ Jour. Amer. Med. Assoc., 1918, 71, 1127.

⁷ Amer. Jour. Med. Sci., 1917, 154, 648.

⁸ Proc. Soc. Exper. Biol. and Med., 1916, 13, 134.

⁹ Jour. Immunology, 1918, 3, 345.

¹⁰ Amer. Review of Tuberculosis, 1918, 2, 523.

Fleischer-Ives Antigen.—Young cultures are removed from a solid medium with saline and centrifuged or filtered through paper. Partially dried bacterial residue is weighed and gradually rubbed up in a mortar with sufficient saline solution to yield a 0.5 per cent. suspension; sterilized and preserved by adding one-tenth volume of 5 per cent. phenol.¹

Non-specific Reactions in Syphilis.—Since tubercle bacilli contain unusually large amounts of lipoids, it is possible that non-specific reactions may occur with the serums of actively syphilitic individuals yielding strongly positive Wassermann reactions, but showing no clinical evidence of tuberculosis. I have studied all of the above described antigens for this property and found that all of them are capable of yielding weakly positive reactions with the serums of syphilitics yielding strongly positive Wassermann reactions. This is especially true if the antigen is employed in an amount corresponding to one-half of the anticomplementary unit. For this reason I thoroughly extract the bacilli with fat solvents before preparing antigen and do not use more than one-quarter of the anticomplementary amount for conducting the test; under these conditions non-specific reactions caused by syphilis reagin are avoided.

Practical Value of the Complement-fixation Test in Tuberculosis.—It is still too early to express an accurate opinion of the practical value of this test; an analysis of numerous reports and my own studies apparently warrants the following statements:

1. A definitely positive reaction taken in conjunction with other findings makes the diagnosis of tuberculosis certain and may be of distinct aid in the diagnosis of early tuberculosis.

2. From the standpoint of differential diagnosis the test is of value when positive as indicating tuberculosis against other diseases of the lungs, as carcinoma, syphilis, abscess, empyema, etc.

3. As the reaction is likely to be positive only in active cases, the complement-fixation test is superior to other biologic tests for active tuberculosis, as, for example, the skin test, which is likely to be positive in persons with quiescent lesions.

4. A persistently and strongly positive reaction probably indicates the presence of active tuberculosis.

5. The strength of a positive reaction appears to bear some relation to the severity of the disease, and reactions becoming gradually weaker until negative have been frequently noted with clinical improvement and "cure."

COMPLEMENT FIXATION IN PERTUSSIS

Investigations on complement fixation with the bacillus of pertussis has given varied results, perhaps due in part to a variation in the cultures employed and the methods used to prepare the antigens.

Bordet and Gengou² used suspensions in salt solution of growths on solid media. Arnheim³ found negative results with antigens of both the Bordet-Gengou bacillus of pertussis and the influenza bacillus. Wollstein⁴ used three forms of antigen: suspensions of the bacilli in salt solution; extracts of bacilli made by suspending the growths of three blood-agar slants in 5 c.c. of salt solution and shaking for twenty-four hours in thermostat; and extracts of tissue obtained from patients dying from pertussis.

¹ Jour. Lab. and Clin. Med., 1918, 3, 302.

² Ann. de l'Inst. Pasteur, 1906, 20, 731.

³ Berl. klin. Wchn., 1908, xlv, 1453; Arch. f. Kinderh., 1909, 1, 295.

⁴ Jour. Exper. Med., 1909, 11, p. 41.

Friedlander and Wagner¹ used live bacteria and fresh serum, and considered this innovation of great importance. The different hemolytic systems employed may also have had some bearing on the failure to obtain identical results.

Bordet and Gengou obtained complement fixation in all of their cases of pertussis. They used 0.1 c.c. to 0.3 of the human serum heated to 56° C., and 0.05 c.c. to 0.01 of fresh guinea-pig serum as complement. The amboceptor was the serum of rabbits previously injected with sheep corpuscles. The antigen was an emulsion of the bacillus of pertussis in salt solution, the growth being twenty-four hours old.

Arnheim² obtained complement fixation in 6 to 12 cases of pertussis. Wollstein examined the serum from 9 patients with pertussis and in no instance did she obtain complement fixation, using the three forms of antigen just mentioned in all cases. The quantities of complement and amboceptor were about the same as those used by Bordet and Gengou.

Gengou and Brunard³ describe 3 cases in which they determined the specific pertussis character of the infection by complement fixation.

In 1911 Bordet and Gengou⁴ reported certain atypical cases diagnosed as pertussis by means of complement fixation. A little later Bordet⁵ concluded that the power to fix complement is not found early, and does not become marked until near the end of the disease.

St. Bächer and Menschikoff⁶ report 27 cases of pertussis in all stages in which attempts were made to obtain complement fixation, without success in a single case. Only after vaccines of pure cultures of the *Bacillus pertussis* were given did fixation occur. Their antigen was an emulsion of the bacillus of pertussis in salt solution, while 0.4 c.c. of a 1 : 10 dilution of guinea-pig serum served as complement, and 0.5 c.c. of a 1 : 150 dilution of serum of a rabbit previously injected with sheep corpuscles served as amboceptor.

Delcourt⁷ obtained complement fixation in 6 cases of pertussis. Poleff⁸ gives a résumé of the results of five investigators. In 5 cases only was there complement fixation and in 31 cases no fixation was obtained. He himself reports 10 cases in which he did not obtain fixation.

Hess⁹ tested 10 cases and concluded "that results would seem to show that this reaction is present for some months after cessation of all symptoms."

Recently Anna Wessels Williams¹⁰ came to the conclusion that the test with serum of human beings is not as clear cut as it seems to be with serum of animals which have been injected with the bacillus of pertussis.

Renaux,¹¹ using the *Bacillus pertussis* for antigen, examined 73 sera for complement fixation, 32 cases of which were known cases of pertussis. Of the cases of pertussis, he obtained complement fixation in 23. Of the 9 negative cases, the serum had been obtained early during the disease, and 3 of these gave positive complement fixation when the attack had lasted about four weeks. No further examination was made on the remaining 6

¹ Amer. Jour. Dis. of Children, 1914, 8, p. 134.

² Berl. klin. Wchn., 1908, 14, p. 1453.

³ Bull. Acad. de med. Belg., 1910, 24, p. 329.

⁴ Centralbl. f. Bakteriöl., I. O., 1911, 58, p. 573.

⁵ Centralbl. f. Bakteriöl., Orig., 1912, 66, p. 275.

⁶ Centralbl. f. Bakteriöl., Orig., 61, p. 218.

⁷ Presse méd. Belg., 1912, 64, p. 19.

⁸ Centralbl. f. Bakteriöl., I. O., 1913, 69, p. 23.

⁹ Jour. Amer. Med. Assoc., 1914, 63, p. 1007.

¹⁰ Arch. Pediat., 1914, 31, p. 567.

¹¹ Centralbl. f. Bakteriöl., I. O., 1914, 75, p. 197.

cases. His results seem to show that fixation appears about three or four weeks after the appearance of the whoop.

In 18 cases of pertussis Friedländer and Wagner¹ obtained complement fixation in each case. They used fresh serum of pertussis patients and living bacteria. The Noguchi hemolytic system was used throughout their work on account of the small quantity of serum necessary. They claim that the diagnosis of pertussis can be made with certainty in the catarrhal stage by means of complement fixation. In a more recent article Friedländer² reports further results. He obtained fixation in 13 of 14 cases in the catarrhal stage before the characteristic whoop had appeared. In one case there was fixation three weeks before the first whoop. Winholt³ has reported positive reactions in pertussis with polyvalent antigens prepared by cultivating the bacilli on potato-glycerin blood-agar, suspending in normal salt solution, and heating to 56° C. for thirty minutes. Olmstead and Povitsky⁴ have been able to differentiate between *Bacillus pertussis* and *B. influenza* by means of the complement-fixation tests employing rabbit immune sera.

Preparation of the Antigen.—Antigens may be prepared in the same manner as gonococcus antigen (page 545) and should be polyvalent. The antigen should always be titrated for the anticomplementary unit as described in the gonococcus complement-fixation test, and used in a dose corresponding to one-third the anticomplementary unit.

Practical Value of the Test.—Positive reactions are not usually obtained until the paroxysmal stage is reached, when about 50 per cent. of sera react positively (Park). For this reason the complement-fixation test possesses little value in early diagnosis. It is at times of value in the diagnosis of atypical cases.

With rabbit immune sera the test has proved of value in the study and differentiation of micro-organisms resembling *Bacillus pertussis*.

COMPLEMENT FIXATION IN OTHER DISEASES

Hog-cholera.—Healy and Smith⁵ have observed positive reactions employing a salt solution extract of mesenteric glands of cholera hogs as antigen; King and Drake⁶ have reported a large percentage of apparently specific reactions employing antigens of *Spirocheta hyos*. At the present time the value of complement-fixation tests in the diagnosis of hog-cholera cannot be stated and the subject requires further investigation.

Acute Anterior Poliomyelitis.—Freese and myself⁷ have observed a small percentage of positive reactions with sera employing salt solution extracts of poliomyelitic brain and spinal cord for antigens. Neustaedter⁸ has reported as high as approximately 90 per cent. of sera of acute cases of poliomyelitis yielding positive reactions with an antigen prepared by digesting an extract of poliomyelitic monkey brain and spinal cord. The subject requires further investigation and may be placed on a practical diagnostic basis.

Vaccinia, Smallpox, and Chickenpox.—In 1906 Jobling⁹ reported positive complement-fixation reactions using as antigen a suspension of finely ground calf vaccine pulp and the sera of vaccinated calves. As far as I am aware this work constitutes the first investigation of its kind conducted in this field and, indeed, was in progress at the time of the original comple-

¹ Amer. Jour. Dis. of Children, 1914, 8, p. 134.

² The Lancet-Clinic, 1915, 113, p. 8.

³ Jour. Infect. Dis., 1915, 16, 389.

⁴ Jour. Med. Research, 1916, 33, 379.

⁵ Jour. Infect. Dis., 1915, 17, 213.

⁶ Jour. Infect. Dis., 1916, 19, 46.

⁷ Jour. Immunology, 1917, 2, 327.

⁸ New York State Jour. Med., 1918, 18, 328.

⁹ Jour. Exper. Med., 1906, 8, 707.

ment-fixation work of Wassermann and his co-workers and Detre in syphilis. In 1909 Beintker¹ reported indifferent results in which he used as antigen a salt solution extract of calf lymph and the sera of vaccinated rabbits and calves as well as the sera of persons suffering with smallpox. In the same year Sugai,² using as antigens salt solution extracts of the contents of smallpox pustules and calf lymph, reported positive reactions with the sera of 6 smallpox patients and 5 persons who had been vaccinated with calf lymph. He found no evidence of agglutination in an extract of calf virus material by these sera. Dalm³ reported positive results with the sera of 10 persons suffering with smallpox and an antigen of calf lymph; he also used watery extracts of the liver and spleen of a two-year-old child who had succumbed to smallpox, but with negative or indifferent results; this investigator also found agglutination of calf lymph by the sera of smallpox patients to be weak or entirely absent. Kryloff,⁴ using a salt solution extract of variola matter, reported positive reactions in variola and varioloid and considered the complement-fixation test as possessing diagnostic value. Aqueous and alcoholic extracts of variolous scabs and the liver and spleen of persons dead of variola as well as salt solution extracts of calf lymph yielded negative or weaker reactions. Bermbach⁵ tested the sera of animals before and after vaccination and the sera of vaccinated and revaccinated persons with a salt solution extract of lymph and reported generally positive reactions. Xylander⁶ tested 31 sera of persons before and after vaccination with an alcoholic extract of lymph in an effort to study the specificity of the Wassermann reaction in syphilis. Of these, the sera of 8 reacted weakly positive with the lymph antigens, and 7 of these reacted in the same manner with the alcoholic extract of heart; after vaccination 18 reacted weakly positive with the lymph antigen and these included the sera of the same 7 persons who reacted weakly positive with the alcoholic heart antigen prior to vaccination. Teisser and Gastinell,⁷ using as antigen a salt solution extract of calf lymph, reported positive reactions with the sera of 39 persons suffering with variola, the reactions becoming positive about the tenth day of the disease and remaining so for at least thirty days; more prolonged observations were not made. Positive reactions were also reported with the sera of vaccinated rabbits, the reactions becoming positive about the seventh day after vaccination. Recently Klein⁸ has also reported favorable results with the complement-fixation test in variola especially with antigens prepared of the contents of the vesicles and pustules of variola and believes the test to possess practical diagnostic value. Konschegg⁹ has also reported positive reactions in variola with salt solution extracts of the contents of variolous lesions. Chang and Chen,¹⁰ however, in a study employing the serums of 50 small-pox patients and antigens of variolous lymph, observed negative reactions with forty-five serums.

In my work¹¹ antigens for the complement-fixation tests were prepared of the contents of vesicles and pustules and the scabs of vaccinia and vaccine pulp from calves and from the contents of vesicles and pustules and scabs of smallpox patients. With these antigens complement-fixation reactions were conducted with homologous antigens and sera and the immuno-

¹ *Centralbl. f. Bakteriol.*, 1909, 48, 500.

² *Centralbl. f. Bakteriol.*, 1909, 49, 650.

³ *Centralbl. f. Bakteriol.*, 1911, 51, 136.

⁷ *Centralbl. f. Bakteriol.*, 1912-13, ref., 55, 555.

⁸ *Münch. med. Wchn.*, 1914, 61, 2270.

⁹ *Münch. med. Wchn.*, 1915, 62, 4.

⁴ *Centralbl. f. Bakteriol.*, 1911, 60, 651.

⁵ *Centralbl. f. Bakteriol.*, 1909, 48, 618.

⁶ *Centralbl. f. Bakteriol.*, 1909, 51, 290.

¹⁰ *Ztschr. f. Immunitätsf.*, 1921, 31, 18.

¹¹ *Jour. Immunology*, 1916, 1, 59.

logic relationship between vaccinia and variola studied by crossing the antigens and sera in complement-fixation experiments; the results were as follows:

1. The sera of rabbits inoculated with cowpox virus yielded positive complement-fixation reactions with salt solution antigens of cowpox and smallpox viruses in seven to eight days after vaccination.

2. The antibody of cowpox virus in the sera of vaccinated animals showed a distinct and close biologic relationship to the antigen of variola in complement-fixation experiments.

3. Of 13 persons vaccinated with cowpox virus from seven days to ten years previously, and whose sera yielded negative Wassermann reactions, positive reactions with salt solution antigens of cowpox virus were observed with 4, or 22 per cent. The sera of 1 of these 4 persons yielding positive reactions (vaccinated eight, twenty-one, twenty-one, and twenty-four days previously) with cowpox virus, reacted positively with a salt solution antigen of variolous material. The sera of unvaccinated persons reacted negatively with all antigens.

4. Of 17 persons suffering with mild smallpox, the sera of 9, or about 60 per cent., yielded positive complement-fixation reactions with salt solution antigens of variolous and cowpox viruses. While the degree of complement absorption was relatively weak in all instances, the reactions were generally stronger with the variolous antigens than with the cowpox antigens.

5. Alcoholic extracts of variolous and cowpox viruses possessed little or no antigenic sensitiveness.

6. These complement-fixation reactions have demonstrated the close biologic relationship between the antibodies of vaccinia and variola; it is probable that complement-fixation reactions with salt solution antigens of the contents of smallpox lesions or fresh cowpox virus will prove of some value in the diagnosis of smallpox.

Girard¹ has observed specific reactions in varicella and believes that the complement-fixation test possesses diagnostic value for differentiating between this disease and smallpox.

Echinococcus (Hydatid) Disease and Intestinal Helminthiasis.—Several investigators have employed a complement-fixation technic in the diagnosis of echinococcus disease of the liver. Ghedini² found this test of value, and he also reports specific reactions with the sera of persons infested with *Ankylostoma duodenales* and *Ascaris lumbricoides*.³ Weinberg,⁴ Jiani,⁵ Israel,⁶ and Henius⁷ report favorable and specific reactions in echinococcus disease with aqueous or alcoholic extracts of cyst fluid, or both. Kurt Meyer⁸ found these reactions non-specific, in that the serum of a person infested with echinococcus showed complement absorption with antigens of *ænia solium* and *T. saginata*, and vice versa. Branes⁹ found that alcoholic extracts of echinococcus cyst fluid showed complement absorption with the syphilis antibody as well as with that of echinococcus disease, and this

¹ La Reaction de Deviation Dans La Varicelle, 1918, Jouve & Co., Paris.

² Gaz. degli Ospedali e delle Cliniche, 1906, 27, p. 1616; 1907, 28, p. 53.

³ Ibid., 1907, 28, p. 476.

⁴ Ann. de l'Inst. Pasteur, 1909, 23, p. 472 (in which references are given to his previous work in this field).

⁵ Wien. klin. Wchnschr., 1909, 22, p. 1439 (in which the author gives a good bibliography of earlier literature).

⁶ Ztschr. f. Hyg. u. Infektionskrankh., 1910, 66, p. 487.

⁷ Deutsch. med. Wchnschr., 1911, 37, p. 1212.

⁸ Berl. klin. Wchnschr., 1910, 47, p. 1316.

⁹ Münch. med. Wchnschr., 1911, 58, p. 1073.

observation has been generally confirmed. Thomsen and Magnusson,¹ in a study of 12 cases of echinococcus disease, found that the sera of 10 reacted positively; the sera of 55 control cases (32 of which reacted positively to the Wassermann reaction) all were negative except one. These authors also report favorably on the specificity of the reaction: the sera of 10 persons infected with *ænia saginata*, of 2 with *T. solium*, and of 1 with *Bothriocephalus latus*, all were negative with echinococcus antigen. Zapelloni² has made a particularly exhaustive study compiling from literature and his own experience the results observed with about 700 cases; about 93 per cent. of sera gave positive reactions, while controls including the sera of individuals with tapeworm reacted negatively.

In so far as echinococcus disease of the liver is concerned, a review of the literature shows a general consensus of opinion that antibodies are present in the sera of the majority of diseased persons and animals and that these may be detected by means of a complement-fixation test. There is, however, a division of opinion in regard to the specificity of the reaction and its practical value in diagnosis.

Much less work has been reported on complement fixation with sera of persons infested with such parasites as *Tænia saginata*, *Ascaris lumbricoides*, etc. Miss Trist and myself³ have observed positive reactions with sera of dogs infested with various parasites and salt solution extracts of the respective worms. The method is worthy of trial in the diagnosis of infestments of persons with the various intestinal parasites. Positive reactions have also been reported by Jerlov⁴ and Fairley⁵ in human cases.

The *antigen* is best prepared of the fresh fluid of an echinococcus cyst of man or sheep or by extracting the thoroughly ground-up parasites with saline solution for other forms of helminthiasis. It should be filtered, if necessary, preserved with 0.5 per cent. phenol, and kept constantly at a low temperature. It is highly important not to use the antigen in an anti-complementary dose, hence it should be titrated before each test is made. I have found that these antigens react positively with syphilitic sera; hence they should be freed of lipoids or used very cautiously if the patient is luetic.

Sporotrichosis and Blastomycosis.—Cases of transmission of sporotrichosis of horses to persons have been recorded, the slowly growing granulomata ending in degeneration being often mistaken for tuberculosis or syphilis; the disease is particularly prevalent among horses in the western states. Several strains of pathogenic sporotricha have been described, but are grouped by Meyer and Aird⁶ as one species, designated as *Sporothrix schenckii-beurmanni*. Moore and Davis⁷ have recently reported favorably upon the specificity and diagnostic value of agglutination and complement-fixation tests, and point out that while antibodies are readily produced in sporotrichosis, they are not generally at all or with difficulty in blastomycosis, which is a closely related disease.

Basedow's Disease.—Recently Berkeley⁸ has reported positive complement-fixation reactions with the serums of individuals with toxic thyroid disease and an antigen prepared of normal thyroid glands of dogs prepared

¹ Berl. klin. Wchnschr., 1912, 49, p. 1183.

² Policlinico, Rome, 1915, 22, 748, 1569.

³ Jour. Infect. Dis., 1916, 18, 88.

⁴ Ztschr. f. Immunitätsf., 1919, 28, 489.

⁵ Med. Jour. Australia, 1921, 1, 205.

⁶ Jour. Infect. Dis., 1915, 16, 399.

⁷ Jour. Infect. Dis., 1918, 23, 252.

⁸ Proc. New York Path. Soc., 1921, 21, 51.

according to a method described by Koopman.¹ Berkeley believes that the reaction may prove of aid in differential diagnosis.

Cancer.—None of the various complement-fixation methods that have been advocated from time to time in the diagnosis of cancer have proved of practical value. von Dungern² has advocated a method which he claims has yielded 90 per cent. of positive reactions in known cases of cancer. Positive reactions have also occurred in tuberculosis and syphilis, and the reports of various other investigators are somewhat contradictory. Whitman, in a study of 30 cases, found the method highly satisfactory. Lucke³ has found the test of no value at all.

The Complement-fixation Test in the Standardization of Immune Serums.—The technic of complement fixation has also been employed as one means in effecting the standardization of antimeningococcic and antigonococcic serums. Since, however, the amount of complement-fixing amboceptors in a serum is no index to its therapeutic and prophylactic value, a measure of this one factor is not a reliable standard.

The *technic* consists in preparing the antigen and in determining its anticomplementary dose. Whatever this is, one-half to one-quarter this amount is added to increasing quantities of heated immune serum, ranging from 0.001 to 0.1 c.c. Complement and saline solution are added, and after incubating one hour at 37° C. the amount of complement fixation is determined by adding hemolytic amboceptors and corpuscles.

While this titration is one measure of the reaction of the animal used in the immunization, better evidence of the therapeutic value of the serum is obtained by determining the content in bacteriotropins, by testing the serum with the antigen in susceptible animals, or by a combination of all methods.

PROTEIN DIFFERENTIATION BY COMPLEMENT FIXATION

The Determination of An Antigen by Complement Fixation.—In the tests hitherto considered the antigens were known, and the suspected antibodies sought for in the blood-serum or other body fluid. In making the reactions it was necessary to bring the serum to be tested into contact with the antigen specific for the suspected antibody, in the presence of complement, and at a suitable temperature. At the end of an hour the mixture was tested for free complement by adding hemolytic amboceptor and red blood-corpuscles. This order may be reversed, and with a known antibody the suspected antigen may be detected. The antigen to be detected, as in a solution of blood or bacterial extract, is brought into contact with its specific antibody in the presence of complement. At the end of an hour, at a suitable temperature, the mixture is tested as previously for free complement, by adding corpuscles and hemolytic amboceptors. Under proper conditions complement fixation would indicate a specific reaction between the antibody and its antigen, and thus serve to identify the latter.

This method has *clinical applications* similar to those in which the precipitin reaction is used:

1. In the differentiation of blood-stains a solution of the stain constitutes the unknown antigen. By furnishing a known antiserum the antigen is detected, *i. e.*, the animal from which the blood was derived is ascertained.

2. In the recognition and differentiation of meats.

¹ Proc. New York Path. Soc., 1921, 21, 56.

² Münch. med. Wchn., 1912, 59, 65, 1098, 2854.

³ Personal communication.

3. In the detection of bacterial antigens in the blood-serum of patients, or with highly immune serums an unknown bacterial antigen may be identified and the test employed as a means of differentiation among bacterial species.

4. Similar applications of the test may be made in the differentiation of milks, seminal stains, and other albuminous substances.

The complement-fixation test, however, shows biologic relationships in the same manner as the precipitin reaction, only to a finer degree, being a much more sensitive reaction. As in the precipitin reaction the complement-fixation reaction will show the species of animal from which an albuminous substance was derived, but does not differentiate among such products from animals of the same species. For example, the reaction cannot differentiate between an extract of human blood, human seminal stain, or human albuminous urine; it is necessary to identify the stain as blood or as semen or as urine by other means. The complement-fixation test will show that it is a human product.

As compared with precipitin reactions, the complement-fixation test is probably more delicate and reliable and easier of interpretation. The technic of the latter method is, however, more complicated, and the liability to error is greater unless the principles of complement fixation in general are thoroughly understood and the importance of quantitative factors is appreciated.

COMPLEMENT FIXATION FOR THE IDENTIFICATION OF BLOOD-STAINS

The application of the technic of complement fixation to the determination of specific protein antigen, such as human or animal blood, was demonstrated by Gengou in 1902. The principles worked out by him were extensively studied and practically applied by Neisser and Sachs¹ in the forensic differentiation of animal proteins.

Author's First Method.—*Hemolytic System.*—*Complement* is furnished by the fresh serum of a guinea-pig diluted 1 : 20 and used in dose of 1 c.c. (= 0.05 c.c. serum); washed *sheep's corpuscles* are made up in a 2.5 per cent. suspension and used in dose of 1 c.c.; *antisheep amboceptor* should be highly potent, and is titrated after the method previously given (p. 473). In the following titrations and in conducting the main test the hemolytic amboceptor is used in an amount equal to 2 units.

Specific Antiserum.—This is obtained from a rabbit immunized with the protein for which the test is to be made, namely, human or animal blood-serum. In forensic tests it may be necessary to prepare a number of these antisera with the serums of man and the ordinary domestic animals. The technic of immunization is the same as that employed for the preparation of precipitins (p. 323). An antiserum for forensic tests must be sufficiently potent to fix complement with 0.001 c.c. of its antigen. This is determined by a process of titration. If, for example, an antihuman serum is to be titrated, the method of procedure is as follows:

Secure 0.1 c.c. of fresh human serum and dilute 1 : 100 by adding 9.9 c.c. of normal saline solution. Of this dilution, 0.1 c.c. is equivalent to the standard dose of 0.001 c.c. of undiluted serum. The antiserum is heated from 60° to 62° C. for half an hour and diluted 1 : 20 (1 c.c. immune serum plus 19 c.c. of saline solution). Decreasing doses of immune serum are mixed with a constant dose of antigen and complement. At the same time the anticomplementary titration of the immune serum is made by substituting salt solution for antigen. The doses to employ and the results of an actual titration are shown in the following table:

¹ Berl. klin. Wchn., 1906, xliii, 67.

TITRATION OF AN IMMUNE SERUM

TUBE.	ANTI-SERUM, 1:20, C.C.	SERUM ANTIGEN, 1:100, C.C.	COMPLE- MENT, 1:20, C.C.	in- cubated for one hour at 37° C.	ANTI-SHEEP AMBO- CEPTOR, UNITS	SHEEP'S CORPUS- CLES (2.5 PER CENT.), C.C.	RESULTS AFTER INCUBATION FOR ONE AND ONE-HALF HOURS.
1....	1.0	0.1	1	Saline solution, q. s. 2 c.c.; tubes gently shaken and incubated for one hour at 37° C.	2	1	Inhibition of hemolysis.
2....	0.9	0.1	1		2	1	Inhibition of hemolysis.
3....	0.8	0.1	1		2	1	Inhibition of hemolysis.
4....	0.7	0.1	1		2	1	Inhibition of hemolysis.
5....	0.6	0.1	1		2	1	Inhibition of hemolysis.
6....	0.5	0.1	1		2	1	Inhibition of hemolysis.
7....	0.4	0.1	1		2	1	Inhibition of hemolysis.
8....	0.3	0.1	1		2	1	Inhibition of hemolysis; <i>unit</i> .
9....	0.2	0.1	1		2	1	Partial inhibition of hemolysis.
10....	0.1	0.1	1		2	1	Slight inhibition of hemolysis.
11....	1.0	0	1		2	1	Very slight inhibition of hemolysis.
12....	0.8	0	1		2	1	Complete hemolysis.
13....	0.4	0	1		2	1	Complete hemolysis.
14....	0.2	0	1		2	1	Complete hemolysis.
15....	0	0.1	1		2	1	Complete hemolysis.
16....	0	0	1		2	1	Complete hemolysis.

Tube 16 is the hemolytic system control, and shows complete hemolysis; tube 15 is the antigen control, and shows complete hemolysis, as the quantity of serum is too small to exert an anticomplementary influence; tubes 11 to 14 are the tests for anticomplementary action of the antiserum. In the present instance the serum was several months old and the maximum dose of 1 c.c. (= 0.05 c.c. undiluted serum) was very slightly anticomplementary. A fresh serum is practically never anticomplementary in this dosage, but these tubes should, nevertheless, be included in each titration. Tubes 1 to 10 include the antigenic titration, and show that the antiserum is perfectly antigenic in dose of 0.3 c.c. of this dilution (= 0.015 c.c. undiluted serum). *In performing the main test double this quantity, or 0.6 c.c., would be used.*

Caution.—Unless an immune serum is perfectly antigenic in at least 0.02 to 0.03 c.c. it should not be used in this test because 2 units must be used and amounts of rabbit serum greater than 0.06 to 0.08 c.c. may yield non-specific complement-fixation reactions. This is the greatest drawback to the use of the complement-fixation test for medicolegal purposes. I always test the sera of rabbits for non-specific complement fixation before immunization is begun. These tests are conducted with rabbit serum heated to 60° to 62° C. for one-half hour and antigen of fresh or heated human serum in dose of 0.1 c.c. of 1:100 dilution. If undiluted rabbit serum in dose of 0.2 or 0.1 c.c. yields a positive reaction this animal is not used for the preparation of an immune serum.

Each antiserum is tested in a similar manner. In forensic blood tests an antihuman serum is, of course, employed first; if this is negative and it is desirable to determine the source of the blood, other antisera, as that of the ox, horse, dog, etc., are prepared, titrated, and tested with a solution of the blood-stain.

The Blood-stain.—It is first necessary to ascertain that the stain is of blood; this is done by performing the hemin crystal or an oxydase test (p. 321). The stain is then extracted in normal saline solution, as

described on p. 321. A 1 : 1000 dilution is made approximately by so diluting the extract that it just gives a slight opalescence when boiled with a few drops of acetic acid, and a slight foam persists after shaking. Unless it is perfectly clear, it should be filtered.

The Test.—Into a series of six small test-tubes place increasing doses of extract of the blood-stain (antigen), as follows: 0.1, 0.2, 0.4, 0.6, 0.8. and 1 c.c.; add double the titrated dose of antiserum and 1 c.c. of complement (1 : 20), with sufficient salt solution to bring the total volume in each tube up to 3 c.c.

The following *controls* are included:

1. Antigen control: 1.0 c.c. of the blood extract plus 1 c.c. of diluted complement and salt solution.

2. Antiserum control: double the titrated dose plus 1 c.c. of diluted complement and salt solution.

3. Hemolytic control: at this time 1 c.c. of diluted complement and salt solution.

4. Corpuscle control: 1 c.c. of corpuscle suspension and salt solution. The tube should be plugged with cotton.

Shake all the tubes gently and incubate for one hour at 37° C. in a water-bath. Add 2 units of hemolytic amboceptor and 1 c.c. of corpuscle suspension to each tube except the corpuscle control. Shake gently and reincubate for from one to two hours in a water-bath, depending upon the degree of hemolysis present in the controls.

The readings are made at once, and again after the tubes have been allowed to settle in the refrigerator overnight. Inhibition of hemolysis with the smallest dose of blood extract—0.1 c.c. (= approximately 0.0001 c.c. of blood)—indicates that the blood extract is most certainly the antigen for the antiserum employed. Even with the maximum dose of extract—1 c.c. (= approximately 0.001 c.c. of blood)—inhibition of hemolysis serves to show the nature of the blood. With an antihuman serum, for instance, a similar specific reaction would be possible only with bloods of the higher apes.

In making blood tests for medicolegal purposes the antiserum should not only be standardized with a definite dilution of human serum, but the whole test should first be conducted with a known dried human blood-stain, and it must be borne in mind that extreme accuracy in all manipulations is essential.

I prefer this complement-fixation test to the precipitin reaction in the differentiation of proteins, as the readings are sharper and more definite. This test is fully as reliable as the precipitin test, and there is less danger of group reaction.

COMPLEMENT-FIXATION METHOD FOR THE IDENTIFICATION OF MEATS

The technic is essentially similar to that used in the foregoing test. Antiserums are prepared by immunizing rabbits with the serums of various animals, as the ox, horse, dog, cat, or any other animal the presence of whose flesh is to be identified in sausages, bologna, etc. It is not necessary to immunize with an extract of these meats themselves, as the blood or blood-serums will suffice. The technic of immunization is the same as that employed in the preparation of precipitin serums. Each antiserum is titrated with its antigen, as previously described, and is used in double the titrated dose in conducting the main test.

An extract of the flesh to be examined is prepared as described on p. 329. The test is then conducted exactly the same as previously described.

In the following table are shown the method and the results of an actual test, using a dried human blood-stain and the same antiserum as previously directed.

FORENSIC BLOOD TEST

TUBE.	EXTRACT OF BLOOD- STAIN. 1:1000 C.C.	ANTI- SERUM 1:20, C.C.	COMPLE- MENT, 1:20, C.C.	Saline solution to 2 c.c.; tubes shaken and incubated one hour.	ANTI- SHEEP AMBO- CEPTOR. UNITS.	CORPUS- CLES (2.5 PER CENT.), C.C.	RESULTS AFTER ONE AND ONE- HALF HOURS' INCUBATION.
1....	0.1	0.6	1		2	1	Marked inhibition of hemolysis.
2....	0.2	0.6	1		2	1	Marked inhibition of hemolysis.
3....	0.4	0.6	1		2	1	Complete inhibition of hemolysis.
4....	0.6	0.6	1		2	1	Complete inhibition of hemolysis.
5....	0.8	0.6	1		2	1	Complete inhibition of hemolysis.
6....	1.0	0.6	1		2	1	Complete inhibition of hemolysis.
7....	1.0	0	1		2	1	Antigen control: hemolysis.
8....	0	0.6	1		2	1	Antiserum control: hemolysis.
9....	0	0	1		2	1	Hemolytic control: hemolysis.
10....	0	0	0		0	1	Corpuscle control: no hemolysis.

COMPLEMENT-FIXATION METHOD FOR THE IDENTIFICATION OF BACTERIAL ANTIGENS

As a means of diagnosis this test has very limited practical value. It aims to detect, by means of complement fixation with a known antiserum, a soluble bacterial antigen in the blood-serum of a patient. For example, in typhoid fever the patient's serum is mixed with a potent antityphoid serum in the presence of complement. After incubating for one hour at 37° C., amboceptor and corpuscles are added to test for free complement. An absence of hemolysis indicates that complement has been fixed by the antiserum and soluble typhoid antigen in the serum of the patient. As a rule, and for purposes of diagnosis, this order of procedure is reversed: the antigen is furnished and then sought for in the patient's serum, as in the gonococcus fixation test, syphilis reaction, etc.

An immune serum is prepared by immunizing rabbits with increasing doses of an emulsion of the bacteria which we wish to test for in the patient's serum.

The bacterial extract is prepared as follows: Make cultures of the bacteria on slants of agar; wash off a sufficient number with normal saline solution until 20 or 30 c.c. of a heavy emulsion are secured; add 0.4 per cent. of phenol, and shake mechanically with glass beads for twenty-four hours; then heat to 60° C. for four hours, and either centrifuge thoroughly or filter through a Berkefeld filter. The clear filtrate should be preserved in a tightly stoppered bottle in an ice-chest. It is well to titrate this extract for its anticomplementary dose. As a rule, these extracts are free from anticomplementary action until relatively large doses are employed.

The antiserum is heated to 60° C. for half an hour and titrated with 0.01 c.c. of the bacterial extract (0.1 c.c. of a 1:10 dilution) in ten doses, ranging from 0.1 to 1.0 c.c. Double the dose giving complete fixation of complement is used in testing for the bacterial antigen in human serum.

In conducting this test the patient's serum is heated to 55° C. for half an hour, and decreasing doses, ranging from 0.5 to 0.01 c.c., are placed in a series of test-tubes together with double the titrated dose of antiserum. Complement and salt solution are now added, and after incubating for an hour at 37° C., amboceptor and corpuscles are added and the tubes re-incubated. The general technic and controls are the same as those previously described.

The test has some value in special research work, but for practical use it has given way to the agglutination reactions and complement-fixation tests for the detection of antibody with a known antigen.

AUTHOR'S NEW COMPLEMENT-FIXATION METHOD FOR THE IDENTIFICATION OF BLOOD-STAINS, BACTERIA, AND OTHER PROTEINS

The complement-fixation method based upon studies in the standardization of the Wassermann reaction has been applied to the differentiation of proteins as the identification of blood-stains, with considerable success. The method has proved much more sensitive than the precipitin test and superior to the complement-fixation test described above.

Great care, however, must be exercised in the choice of rabbits for the preparation of immune sera because the test is so sensitive that the majority of normal rabbit sera yields non-specific reactions unless amounts smaller than 0.02 c.c. are employed. For this reason I have worked out a method for preparing immune sera by the immunization of guinea-pigs.

The technic embraces the same principles as described above. Owing to lack of space the details based upon extensive investigations covering the selection and immunization of rabbits and guinea-pigs, preservation and titration of immune sera, preparation and titration of antigens (sera, meats, seminal fluid, etc.) are to be given in the author's separate monograph upon the complement-fixation reaction.

CHAPTER XXVI

CYTOTOXINS

IN Chapter XVIII the cytolytins in general were considered, especially their theoretic structure and the mechanism of their action.

It will be remembered that the general name *cytolysin* is applied to an amboceptor or antibody of the third order of receptors that is capable of preparing its antigen for the disintegrative or lytic action of a complement. The two best known and most important members of this group of antibodies have been considered, namely, the hemolysins and the bacteriolysins.

Following the discovery of the hemolysins and the bacteriolysins and of the mechanism of their action, it was not long before similar studies were undertaken with other cells, with the result that attempts have been made to prepare immune cytolytic serums for practically every organ of the body. This outcome was but natural, in view of the enormous theoretic importance of specific cytolytins, not only from the additional light that may be thrown upon physiologic and pathologic processes in general but also from the standpoint of specific therapeutics.

Nomenclature.—While actual lysis or solution of erythrocytes and bacteria may be brought about by antibodies of this order, yet actual solution is not apparent with most other body cells, although a distinct toxic action may be observed. For instance, an antispermatozoa serum will cause these cells to lose their motility, but does not actually dissolve them. Hence the name *cytotoxin* has been applied to these immune serums. This is probably a better term than *cytolysin*; but it is to be remembered that, so far as is now known, both cytolytins and cytotoxins are antibodies that possess the same nature and structure, except that in the former group the process is complete and ends in actual lysis of the cell. The term "*cytolysin*" is, therefore, more appropriately applied to the bacteriolysin and hemolysins; whereas the term *cytotoxin* is reserved for those immune serums that injure their cells without complete lysis (a toxic action), such as nephrotoxin, hepatotoxin, etc. This chapter is mainly concerned with the latter group.

Nature and General Properties of Cytotoxins.—As previously stated, cytotoxins are amboceptors or antibodies of the third order, and possess the same general properties as the bacteriolysins and hemolysins, except that, as will be pointed out later, they do not possess the same specificity. Without the presence of a complement they are inactive. They are thermostable, possess the same general affinity for their antigen, and may be removed from a serum by saturation with the antigen in a manner similar to that used for the removal of a hemolysin.

Natural Cytotoxins.—Human serum as well as the sera of the lower animals may contain small amounts of cytotoxins for a wide variety of cells. Flexner and Noguchi¹ have found cytotoxins in the sera of warm and cold-blooded invertebrates for cells of the kidney, liver, and testes. By absorption tests these were found to possess a well-defined, but not an absolute specificity.

Preparation of Cytotoxins.—Cytotoxic serums are prepared by immunizing an alien animal with fine suspensions of cells of the particular

¹ Jour. Med. Research, 1903, 9, 257.

organ being studied. Every effort should be made to remove all traces of blood, and to secure as pure an emulsion of the same cells and to work as aseptically as possible. Injections are best given intraperitoneally, rabbits being well adapted for the preparation of these serums.

Beebe¹ has made the statement that more specific serums are obtained if the nucleoproteins are isolated and used in the process of immunization, than if the cells themselves are used. Wells,² however, believes that the nucleoproteins of cells are not specific in character, and Pearce, Karsner, and Eisenbrey³ found that nephrotoxic and hepatotoxic serums prepared by the injection of the nucleoproteins of these cells were no more toxic than serums prepared from the globulins and albumins of the same organs.

Methods of Studying Cytotoxins.—While the phenomena of hemolysis and bacteriolysis may readily be observed in experiments *in vitro*, the influence of other cytotoxins on the particular cells used as antigens is more difficult to determine.

Microscopic Method.—Suspensions of cells may be obtained by passing the tissue through a meat grinder and shaking the pulp very vigorously in a large test-tube with sterile saline solution. On standing, the emulsion separates into three layers: a lower layer of large fragments, a medium of separated cells, and an upper in which fragments of cells and blood-corpuscles are contained. The middle layer is removed, again shaken with saline solution, allowed to stand and the supernatant opaque suspension removed, filtered through gauze, and employed.

The serum must be fresh and should be used undiluted; 1 part of suspension of cells may be mixed with 9 parts of serum and incubated at 38° C. for two hours followed by placing in a refrigerator for several hours. The effects of cytolysis may be apparent by partial clearing and by microscopic examination to detect evidences of cytolysis. Controls in which physiologic saline solution replaces serum should always be included.

Animal Inoculation Method.—The technic generally employed consists in making subcutaneous, intraperitoneal, or intravenous injections of the immune serum into the animal, or into the arteries leading to particular organs. Functional disturbances and delicate histologic changes in various organs have served as criteria for determining the degree of specificity that exists. The loss of some manifestation of vitality on the part of the cell, as a loss of motility (spermatozoa) or an inability to proliferate, may aid in studying the effect of these serums.

Complement-fixation and Other Methods.—More recently several other methods have been used, especially the complement-fixation and the epiphanin reactions; Fleischer, Hall, and Arnstein⁴ have employed a complement-fixation reaction with success. Rabbits were immunized with the liver and kidney of the guinea-pig and these sera employed with antigens of the guinea-pig organs prepared by different methods. By means of these reactions and absorption tests they were able to show that there exists a definite relationship between the antiorgan sera and the homologous antigens.

Lambert⁵ has suggested that a method of cultivation of tissues outside of the body may constitute an ideal technic. He has observed that mouse sarcoma, which grows vigorously in the plasma of normal rats, shows little or no activity in the plasma of rats immunized by mouse sarcoma injec-

¹ Jour. Exper. Med., 1905, vii, 733.

² Chemical Pathology, 1914, second edition, W. B. Saunders Co.

³ Jour. Exper. Med., 1911, xiv, 44.

⁴ Jour. Immunology, 1920, 5, 437; *ibid.*, 1921, 6, 223.

⁵ Jour. Exper. Med., 1911, 14, 453.

tions. Rat sarcoma, readily cultivated in the plasma of normal guinea-pigs, remained inactive or presented a feeble growth in the plasma of guinea-pigs previously treated with rat tissues. Lambert has attributed these results to the action of cytotoxins.

Specificity of Cytotoxins.—As has been stated elsewhere, the hemolysins and the bacteriolysins are highly specific, especially the former group. With the cytotoxins, however, this specificity is not observed. Most cytotoxic serums are also hemolytic, notwithstanding the fact that careful precautions have been taken to remove, so far as possible, all traces of blood from the inoculum during the process of immunization. Metchnikoff found a spermatotoxic serum to be also hemolytic, but he believed that this property could be removed by treating the immune serum with the corresponding corpuscles, and in this manner dissolve out the hemolysin. Numerous other investigators have found, however, that cytotoxic serums may attack the cells of other organs, as well as those that have been used as their antigens.

The subject has been very carefully investigated by Pearce.¹ The injection of an antidog nephrotoxic serum prepared by immunizing rabbits with washed dog kidney is followed by the development of a tubular nephritis, with albuminuria and occasionally hemoglobinuria, and accompanied by granular degeneration of the liver. These serums are usually hemolytic *in vitro*. Similarly, in a study of hepatotoxic serums, Pearce found that the most striking lesions were referable to the hemagglutinating and hemolytic properties of the serum, causing thrombosis, embolism, and hemorrhages, whereas secondary necroses may be caused by a direct toxic action of the serum on certain parenchymatous cells.

Pearce, Karsner, and Eisenbrey found that the serums of rabbits injected repeatedly with the nucleoproteins, globulins, and albumins of the liver and kidney of the dog gave no evidence of organ specificity *in vitro* or *in vivo* experiments. These investigators were not able to support the view put forward that nucleoproteins play an important part in the production of cytotoxic immune serums.

Lambert² has recently studied the subject with cultures of rat sarcoma and rat embryo skin and their immune serums, and found that these cytotoxins were not specific for the tissue injected.

These results are not surprising when it is remembered that all the body cells have a common origin, and that, although the cells of various organs may differ considerably in morphologic and functional characters, they have certain receptors in common, and, as Pearce originally maintained, it is hardly conceivable that specific somatogenic cytotoxins can be produced.

That some degree of specificity may exist is, however, to be considered as a possibility. By means of the complement-fixation reaction Fleischer and Arnstein³ have recently observed that it is possible to demonstrate some tissue specificity in liver, kidney, spleen, brain, muscle, and testicle, their experiments being conducted by injecting guinea-pig tissues into rabbits. In some cases they observed absolute specificity, while in others specificity was only relative. The difficulty in demonstrating specificity was found in the extreme complexity of the biologic composition of the tissues, and possibly in the interrelationship existing between various tissues.

Autocytotoxins.—While these toxins possess some theoretic interest, they are of very rare occurrence in experimental work. Certainly cells of

¹ Jour. Exper. Med., 1914, xix, 277 (includes a good review of the literature).

² Univ. Penna. Med. Bull., 1903, xvi, 217; Jour. Med. Research, 1904, xii, 1.

³ Jour. Immunology, 1921, 6, 223.

the kidney, liver, and other organs are constantly dying and being replaced by new cells; the receptors of these cells are thereby set free, and are capable of forming a union with the receptors of other cells, and the possibility for the formation of autocytoxicins is established. According to Ehrlich, however, the side arms anchoring the receptors of the dead cells are sessile in nature, and are unlikely to cause overproduction, as in the case of antibodies for bacterial substances or for the cells of other species. On the other hand, a simultaneous production of anti-autotoxins that counteract the autotoxins and preserve a delicate physiologic equilibrium may occur, the whole subject being, however, still in the experimental stage.

Of most interest in this connection are the theoretic autonephrotoxins. These may be produced when part of a kidney becomes disorganized in the living body, as by means of a toxin. Theoretic autotoxins may then be produced, which, acting upon other kidney cells, institute a vicious cycle. Acting upon these assumptions, Ascoli and Figari¹ and Lindeman² have proposed a new theory as to the pathogenesis of certain of the nephritides. These observers would account for the cardiac hypertrophy of nephritis by attributing it to the action of the nephrotoxic serum in causing contraction of the peripheral vessels, with consequent increase of blood-pressure; the nephritic nervous symptoms, they believe, are due to the fact that the serum contains a neurotoxic constituent.

Lindeman has produced a toxic nephritis in dogs by giving them injections of potassium bichromate, and found that the serum, although free from the chromate, was toxic to other dogs, a finding he believed due to the presence of autonephrotoxins produced in the first dog as a result of the destruction of kidney cells. According to this view, the original toxic cause of a degenerative nephritis would be less responsible for the continuance of the process than would the formation of an autonephrotoxin. While these conclusions are somewhat far-reaching, they serve to indicate that the same processes operative in bacterial infection and immunity may have an important relation to other pathologic conditions.

It is not rarely observed that in large tumors and similar lesions certain groups of cells may undergo digestion, but in these the lysis is commonly ascribed to the action of ferments liberated upon the death of cells.

Isocytotoxins have been produced experimentally, as, for example, by Ehrlich, who produced isohemolysins by injecting goats with goat blood, and by Metchnikoff, who prepared isospermatoxic serums.

Anticytotoxic serums have likewise been prepared by careful immunization with cytotoxic serums. They were first discovered by Camus and Gley³ and independently of them, by Kossel⁴ in connection with a study of the toxic power of blood-serum of eels. Animals treated with eel's serum acquire an antitoxic property which protects their corpuscles against the hemolytic action of ichthyotoxin, or the toxic substance of the blood of eels.

Varieties of Cytotoxins.⁵—As previously stated, attempts have been made to prepare cytotoxic serums for practically all the organs and tissues. Since none of these has been found to be absolutely specific, and hence since they possess little or no practical value, they will receive here but brief consideration.

1. *Spermatotoxin*.—This serum was prepared simultaneously by Metch-

¹ Berl. klin. Wchn., 1902, xxxix, 634.

² Ann. de l'Inst. Pasteur, 1900, xiv, 49.

³ Archiv. internat. d. Pharmacol., 1898, 3 and 4.

⁴ Berl. klin. Wchn., 1898, 152.

⁵ For literature see Sachs, Biochem.-Centralbl., 1903, 1, 573, 613, 653, 693.

nikoff¹ and Landsteiner in 1899, and was one of the earliest cytotoxins to be studied. It is a hemolytic serum, and causes spermatozoa to lose their motility. As shown by Metchnikoff² the preparation of these sera is frequently difficult. Furthermore, their full activity was only apparent after the sera had been heated to 56° C. It would seem to affect also the vitality of the spermatozoa *in vivo*, inasmuch as De Lester, by the injection of this serum, rendered male mice sterile for from sixteen to twenty days.

Dittler³ has recently renewed interest in this subject by finding that immunization of female rabbits with rabbit sperm renders them sterile for a few months. Immunization with sperm from other species failed to produce sterility, and as the serum of the rabbits injected with rabbit sperm was spermatotoxic and agglutinative, Dittler believes that the temporary sterilization depends on immunization against the sperm of the homologous species. These and similar observations have aroused considerable speculation regarding their possible relation to sterility.

2. *Epitheliotoxin*.—A cytotoxic serum for the ciliated epithelium of the trachea was prepared by von Dungern.⁴ The cells became disintegrated in the peritoneal cavity of the immunized animal, but not in that of the normal animal. This serum also proved to be hemolytic.

Similar serums have been prepared with cancer cells, in the hope of establishing a specific serum therapy, but all efforts have thus far proved futile.

3. *Leukotoxins*.—This serum was first prepared by Metchnikoff⁵ and Besredka⁶ by injecting the spleen of rats into guinea-pigs. The serums have also been prepared by effecting immunization with exudates rich in leukocytes or with the emulsion of lymphoid organs (Flexner and Ricketts). They are usually hemolytic, and also attack endothelial cells. Their action may be observed *in vitro* when the leukocytes lose their ameboid motility and the protoplasm swells, clears, and may disintegrate, leaving the nucleus.

Lindstroem⁷ has recently prepared a serum by injecting a sheep intravenously with rabbit leukocytes and reports that the administration of this immune to 3 cases of myeloid leukemia had a profound influence upon the leukocytes and produced temporary benefit.

4. *Nephrotoxin*.—This serum is best adapted for experimental studies of the cytotoxins. As shown by Pearce, with the aid of this serum physiologic and anatomic alterations and lesions are readily studied. The injection of a nephrotoxic serum produces a tubular nephritis, with albuminuria and possibly hemoglobinuria. The serums are usually hemolytic, and frequently cause degenerative lesions in the liver, due in part to hemolysis and hemagglutination of red corpuscles.

5. *Hepatotoxin*.—Delezenne⁸ was the first to work with the so-called hepatotoxin, which, he claimed, possessed absolute organ specificity. Subsequent investigations, however, have brought forth contradictory findings. Pearce found that hyaline thrombi, formed of agglutinated red corpuscles, are primarily responsible for the areas of necrosis and hemorrhage, with secondary effects, which may be ascribed to a cytotoxin liberated chiefly by the cells in the thrombus, and acting on the liver cells. These findings and views have recently received support from the investigations of Karsner and Aub.⁹

6. *Gastrotoxin*.—Gastrotoxic serums have been studied by Bolton,¹⁰ who

¹ Ann. de l'Inst. Pasteur, 1900, xiv, 369.

² Ibid., 1900, 14, 5.

³ Münch. med. Wchn., 1920, 67, 1495.

⁴ Münch. med. Wchn., 1899, xlv, 1228.

⁵ Ann. de l'Inst. Pasteur, 1899, xiii, 737.

⁶ Ann. del 'Inst. Pasteur, 1900, xiv, 402.

⁷ Arch. d. mal. du cœur, 1921, 14, 145.

⁸ Semaine méd., 1900, xx, 290.

⁹ Jour. Med. Research, 1913, xxviii, 377.

¹⁰ Proc. Roy. Soc., lxxvii, 426, and lxxix, 533.

immunized rabbits with emulsions of the mucosa of the stomach of the guinea-pig. The injection of this serum into guinea-pigs was followed by the development of areas of hemorrhage, necrosis, and ulcer formation that resembled peptic ulcers. According to Bolton, if the gastric secretions were neutralized with large quantities of alkali, the ulcers did not develop, indicating that the peptic ferments may be operative in the digestion of the cells after their destruction by the immune serum. Gastrotoxic serums were found to produce precipitates with clear filtrates of gastric cells, and were also shown to be hemolytic.

7. *Synocytotoxin*.—This serum has been produced experimentally by immunization with an emulsion of placental cells. According to Liepmann,¹ it produces a precipitate with a filtrate of placenta cells, and at one time it was believed that it might constitute a diagnostic test for pregnancy.

Syncytotoxins are interesting as considered in reference to eclampsia and other toxemias of pregnancy. As is well known, placental cells may become detached and, gaining entrance to the circulation, become lodged in remote organs (Schmorl). This has given rise to the theory that a placentotoxin is developed that produces the nephritis of pregnancy and necrotic lesions in the liver. Weichardt asserts that, by digesting placenta *in vitro* with an active placentotoxic serum and injecting the digestate, he produced symptoms resembling eclampsia in the lower animals. It was hoped that an anticytotoxic serum might be prepared to combat the effects of the placentotoxin, but this hope has not been realized. Renewed interest in this particular subject has been manifested by the recent studies of Abderhalden in ferments as applied to the diagnosis of pregnancy (p. 231).

8. *Neurotoxin*.—This toxin has been prepared and studied by Delezenne,² Centanni, Delille, and others by immunization experiments with emulsions of cerebrum, cerebellum, and spinal cord. When injected into the brain direct these serums may cause profound intoxication of the nerve-centers, with torpor or convulsions, subnormal temperature, and death. When injected directly into the veins they are usually without effect. In addition to their neurotoxic action they are generally hemolytic, and frequently endotheliotoxic and leukotoxic.

9. *Thyrotaxins*.—This serum is prepared by immunizing animals with emulsions of thyroid gland. Thyrotaxins were quite prominently before the profession a few years ago, owing to the work of Beebe,³ who advocated their use in the treatment of various goiters. They have not fulfilled their expectations, however, since they may also produce degenerative changes in the various organs, as the liver, spleen, and kidneys.

Adrenotoxins.—In 1901 Bigart and Bernard⁴ found that the serum of ducks which had been injected with the adrenals of guinea-pigs was capable of killing these animals and that the medullary portion of the adrenals was sometimes diffuent and gelatinous. Abbott,⁵ Pearce,⁶ and others, however, have failed to produce adrenotoxic sera by the immunization of rabbits. Ritchie⁷ has reported the successful preparation of a serum by immunization of ducks, which was adrenophilic, but not adrenolytic. This serum was not strictly specific, but there was no evidence of it being hemolytic.

Thymotoxins.—Ritchie⁸ has immunized ducks with suspensions of cells

¹ Deutsch. med. Wchn., 1902, xxviii, 911.

² Ann. de l'Inst. Pasteur, 1900, 14, 686.

³ Jour. Exper. Med., 1905, 7, 732.

⁷ Jour. Path. and Bacteriol., 1908, 12, 140 (gives a good bibliography of the literature on

the cytotoxins in general).

⁸ Jour. Path. and Bact., 1908, 12, 140.

⁴ Compt. rend. Soc. de biol., 1901, liii, 161.

⁵ Jour. med. Res., 1903, 49, 329.

⁶ Jour. med. Res., 1904, 12, 1.

from the thymus glands of guinea-pigs. These sera were leukophilic, but not hemolytic. Structural changes were produced by injecting these sera into guinea-pigs, but these were ascribed to its leukolytic action and were regarded as non-specific.

10. *Infusoriotoxins*.—Rossle¹ and more particularly Takenouchi² have described toxins for various infusoria in normal and immune sera. Takenouchi has studied human, horse, sheep, hog, beef, guinea-pig, rabbit, pigeon, frog, and turtle sera with paramecia finding the evidences of toxic action expressed by depression of motility, discharge of trichocysts, swelling, and finally disintegration. Toxic activity was found to disappear when serum was heated. Curiously, the sera of cold-blooded animals were found much more toxic than the sera of warm-blooded animals.

RÔLE OF CYTOTOXINS IN IMMUNITY

It is apparent that, according to our present knowledge, the cytotoxins proper, although they possess great theoretic importance from their possible relationship to the removal and disposal of enfeebled and dead cells, occupy a subsidiary place in the processes of immunity. The processes governing these changes are finally and delicately balanced, and although obscure, they offer an intricate but fascinating field for research.

If the ferments concerned in Abderhalden's studies are related in any way to the cytolytins, the subject becomes of great interest, and a new field, with immense possibilities, is opened for further study.

Practical Applications.—(1) In *therapeutics* the cytotoxins have not established a place for themselves and their use has been disappointing. As previously stated, the use of thyrotoxic serums has not met with considerable success; epitheliotoxic serums have likewise not been efficient in the treatment of cancer. They possess theoretic interest, however, from the possibility of their so injuring glands that their functions may be studied; theoretically, the use of minute and carefully graded doses of hemolytic serums may effect the production of antihemolysins and aid in the treatment of certain anemias.

(2) In *diagnosis* cytotoxic reactions have been employed by Freund and Kaminer. These observers used the cytotoxins as a diagnostic aid in cancer, but with indifferent success.

CYTOTOXIC REACTIONS

Cytolytic Cancer Diagnosis of Freund and Kaminer.³—This reaction is based upon the observation that while normal serum has the power to dissolve cancer cells, the serum of cancerous persons lacks this property, and has the power to inhibit the destruction of such cells by normal serum.

The same authors have also observed that when cancer serum is mixed with an extract of cancer cells a precipitate forms. They claim to have secured 88 per cent. of positive reactions in 113 cases examined, and believe that the reaction occurs early enough and is sufficiently specific to render it of practical value. These observations, however, have not been sufficiently confirmed.

An *emulsion of cancer cells* is prepared by grinding the undegenerated portions of a tumor, freed as much as possible from fat and fibrous tissue, in a mortar and adding about five volumes of 1 per cent. sodium biphos-

¹ Arch. f. Hyg., 1905, 54, 1.

² Jour. Infect. Dis., 1918, 23, 396.

³ Biochem. Ztschr., 1910, 26, 312; Wien. klin. Wchn., 1910, 23, 378, 1221; *ibid.*, 1911, 24, 1759.

phate. The suspension is filtered through several layers of gauze, and after the cells have become precipitated, the supernatant fluid is decanted. The residue of cells is washed with 0.6 per cent. sodium chlorid and allowed to settle again, the supernatant fluid is decanted, and the residue covered with 1 per cent. sodium fluorid. The last-named fluid must first be neutralized against alizarin until only a trace of the violet color remains. The emulsion will keep for several weeks in an ice-chest.

Serum.—The patient's serum should be collected just a few hours (not over twenty-four) before the test is to be made, and must be clear and free from cellular elements.

The Test.—To 10 drops of the patient's serum add 1 drop of 0.5 per cent. solution of sodium fluorid. Then add 1 drop of the cancer-cell emulsion so diluted that when 1 drop of the mixture is placed in a blood-counting chamber, about 10 to 20 cancer cells will be found in a field of four large squares. Close the counting chamber carefully, ring with vaselin to prevent evaporation, and place in the incubator for twenty-four hours.

A second slide is prepared from a mixture composed of one volume each of normal serum, cancer serum, and 0.6 per cent. sodium chlorid and sufficient cell emulsion.

A third slide is prepared with a fresh normal serum in the same manner as when the patient's serum is used.

All slides are incubated for twenty-four hours at 37° C. and the cells counted. A material reduction in the number of cells with the normal serum will be noted; if the patient has carcinoma, the first and second slides will not show this reduction, whereas if the patient is free from cancer, similar reductions will be found in all three slides.

The authors recommend that both the cytolytic and the precipitin test be conducted when enough serum is available for both. Herly¹ has recently applied this test to a study of the serums of normal rats and rats inoculated with the Flexner-Jobbing rat carcinoma. Normal rat serum was found without any deleterious effects upon the cells of this tumor; likewise the serums of tumor rats did not show any effects.

PRODUCTION OF CYTOTOXINS

The term "cytotoxins" is usually applied to cell toxins other than hemolysins, such as nephrotoxins, spermatotoxins, etc., and although "lysin" is frequently used, the term "toxin" is better, being descriptive of the changes produced by all cell toxins except the hemolysins and the bacteriolysins.

Cytotoxic serums can be made theoretically for any cell, but only the hemolysins possess much practical value. The cytotoxins are prepared with some difficulty by injecting emulsions of cells from one animal into another. Immunization should always be conducted by means of intraperitoneal or subcutaneous injection unless the material is so finely divided that intravenous injection is a safe procedure.

Antispermatozoa serum is prepared by injecting rabbits intravenously with 1 c.c. semen diluted with 4 c.c. sterile saline solution every five days; after five or six injections the rabbit-serum is usually found to contain large amounts of precipitin, complement-fixing and cytolytic amboceptors, suitable for the detection of seminal stains. The animals should be bled about ten days after the last injection.

For the purpose of studying the action of cytotoxic serum, nephrotoxic

¹ Jour. Cancer Research, 1921, 6, 337.

serum is preferably to be used, as the effects, *e. g.*, the production of albuminuria, may be observed. A series of two or three animals should be carried along at the same time, as many die after the third injection. So far as possible an aseptic technic should be carried out.

Practically all cytotoxic serums are hemolytic partly because of the great difficulty of removing all traces of blood from the organ used in preparing the emulsion. This difficulty may be reduced to a minimum by thoroughly washing the organ or tissue prior to preparing an emulsion for injection.

The following method, after Pearce, illustrates the mode of preparing a nephrotoxic serum by immunizing rabbits with dog kidney:

1. Anesthetize a dog with ether, open the abdomen, wash the blood out of the kidneys by inserting a cannula high up in the abdominal aorta, and flushing with from 6 to 10 liters of salt solution; open the vena cava.

2. Remove the kidney as aseptically as possible, and grind it in a meat-grinder; rub through fine-meshed wire gauze, and wash the residue in several changes of sterile salt solution. The fat should be rejected and only the cortex used.

3. Weigh and suspend 10 gm. of the substance in 30 c.c. of sterile salt solution.

4. Inject the rabbit intraperitoneally with 10 c.c. of the emulsion every seven days.

5. After the third dose has been given test the serum, as subsequent doses increase the danger of losing the animal.

6. The animal is bled one week after receiving the last injection.

7. The injection of this serum into dogs is usually followed by albuminuria and possibly hemoglobinuria. This subject is further considered under the head of Practical Exercises with Cytotoxins.

Some investigators have asserted that by effecting immunization with the nucleoproteins of an organ more specific cytotoxic serums are secured. These claims have not been confirmed by Pearce, Wells, and others.

Nucleoproteins may be secured as follows: Grind the organ or tissue in a meat-grinder, and finally rub up with sand with a pestle in a mortar; add two volumes of normal salt solution, and pass through a meat press; collect the effluent, place in a refrigerator for twenty-four hours, and then filter through gauze and centrifuge the filtrate; to the supernatant fluid add acetic acid to remove the nucleoproteins. Place in the refrigerator for eighteen hours and centrifuge. Collect the sediment and wash several times with normal salt solution. Dissolve the sediment in normal salt solution containing 0.5 per cent. sodium carbonate. Reprecipitate with acetic acid, wash, and redissolve in the alkaline solution.

CHAPTER XXVII

THE RELATION OF COLLOIDS AND LIPOIDS TO IMMUNITY

WHILE at the present time Ehrlich's side-chain theory best explains the specificity and mode of action of various antibodies, there is a growing tendency to explain many of these reactions on a physicochemical and colloidal basis.

From the fact that, without exception, antigens are colloids, and that antibodies also are colloid in their chemical characters, it is advisable to review briefly some of the main facts and theories concerning these bodies and their reactions.

Varieties of Colloids.—Colloids may be composed of two different classes of substances:

1. *Organic* substances, as, *e. g.*, all forms of proteins and also gums, starch, glycogen, tannin, chondrin, the greater number of organic dyes, and probably the enzymes.

2. *Inorganic* substances, as, for example, the inorganic colloids, such as silicic acid, ferric hydroxid, arsenic sulphid, and many other similar compounds.

Since the living tissues and fluids are, without exception, colloids and colloidal solutions, the properties of the cells are largely the properties of colloids.

Nature and Properties of Colloids.—Since Graham,¹ in 1861, studied the differences between the substances that did or did not diffuse readily through animal or parchment membranes, soluble substances have been classified in two main groups: (*a*) *Colloids*, or those substances that were dissolved to the extent of showing no visible particles in suspension, but that did not pass through diffusion membranes at all, or did so very slowly indeed, and (*b*) *crystalloids*, or solutions that diffuse through membranes quite readily.

Since Graham's discoveries investigations have shown that any and all substances may be either colloid or crystalloid, depending upon the treatment they receive; thus albumin may be crystallized and common table salt obtained in a state of colloidal solution.² Furthermore, there is much evidence indicating that all colloid systems are unstable and never in equilibrium; conditions which determine the appearance of a body in the colloid or crystalline form appear to indicate that bodies always separate from solution in the amorphous or colloidal condition and that ell crystallization is a secondary phenomenon.

On the other hand, we may have substances that are quite insoluble when aggregated in masses, but when derived in pure form by mechanical means can be suspended and uniformly distributed through a fluid without showing any marked tendency to precipitate. Such *suspensions* or *emulsions* contain particles that are visible under the microscope; they usually appear turbid, do not transmit electricity, and are not diffusible. *Colloids occupy a place between the true solutions of crystalloids and the emulsions.* Sharp boundaries cannot usually be drawn between any of the members of

¹ Phil. Trans., 1861, 183.

² V. Weimarn, Ztsch. f. Chem. Ind. Koll., 1908, 326; *ibid.*, 9110, 7, 92.

the series. They differ quantitatively in some manner from the true solutions and the emulsions, but may approach them closely, and sometimes resemble them so strongly as to be almost indistinguishable from them. For the most part, however, they show decided characteristics that will differentiate them from the crystalloids, on the one hand, and the suspensions, on the other.

Those colloids that closely resemble the true solution have been designated *colloidal solutions*, and those resembling more closely the suspensions, *colloidal suspensions*. Of the two types, the colloidal solutions are far more important biologically, since the colloidal suspensions are usually prepared artificially and seldom occur in nature.

Colloids, therefore, appear to be suspensions of masses of molecules, or perhaps of very large single molecules. When these aggregations are sufficiently large, we have an ordinary suspension.

When colloids occur in a highly dispersed state they are called *sols*; when in an undispersed or but slightly dispersed state they are spoken of as *gels*. A colloid substance may be converted from a *sol* state into a *gel* state and back again, when it is called a *reversible* colloid or emulsion; a colloid which refuses to redisperse is an *irreversible* colloid or suspension.

1. *Colloids are usually amorphous in character*, and with few exceptions do not present a typical structure; they are not crystalline under any visible condition. This, however, is not invariably the case, for we may have a protein, like hemoglobin, which resembles a typical colloid in every respect, and may yet form crystals readily and abundantly.

2. *Colloids do not form true solutions*, but the solvent is probably an important factor in determining whether or not a substance is colloidal in nature; *e. g.*, soaps form true solutions in alcohol and colloidal solutions in water; rubber forms colloidal solutions in ether, but not in water. The term "colloidal solution" does not, therefore, refer to a true solution in the sense of a crystalloid, but to a colloidal state of suspension (the so-called colloidal solution).

3. *Colloids are non-diffusible*, or lack the power of passing through animal and parchment membranes. Not all colloids possess the same rate of diffusion, this property being relative rather than absolute; however, solutions of salts (crystalloids) pass through so readily that they are easily separated from proteins (colloids) by dialyzation, a process that is in constant practical use.

4. *Colloids have an extremely small osmotic pressure*. They may, to a very slight degree, exert some influence upon osmotic pressure, the freezing- and boiling-points of fluids, but in all cellular processes in which manifestations of osmotic pressure or diffusion are present the crystalloids may be considered as almost entirely responsible for these.

5. *The colloids exhibit surface tension to a high degree*—in other words, colloid fluids possess the force that strives to reduce its free surface to a minimum. As partial expressions of this force, the formation of emulsions when oil and water are mixed and the ameboid movements of the ameba and leukocytes may be mentioned as examples.

6. *Colloids do not separate freely into ions when dissolved, and accordingly do not conduct electricity to an appreciable extent*. When an electric current is passed through a colloidal fluid, most of the colloids move toward the anode; this phenomenon, known as cataphoresis, is also generally exhibited by suspensions, and in this particular the colloids resemble suspensions.

7. *Colloids are usually easily precipitable and coagulable*, and this is

readily understood when the slender margin that exists between many of the colloids and the suspensions is borne in mind. Relatively slight changes, such as exposure, gentle heat, the presence of large quantities of crystalloids, the action of enzymes, etc., may throw an organic colloid out of solution, and when once precipitated, it is often incapable of again dissolving in the same solvent. Colloids are also precipitated by many electrolytes, apparently through the formation of true ion compounds.

8. *The physical structure and size of colloids.* This subject has been studied extensively by Hardy.¹ Cells contain but one type of colloids, the proteins that form non-reversible coagula. So long as a colloid is in solution it is structureless; but such solutions may become solid as the result of changes of temperature and other physical means and from admixture with certain chemical fixing agents. The structure of the coagula varies according to the concentration of the colloidal solution and the nature of the coagulant, but in general the figures obtained in the solidification of protein solutions by such fixing agents as mercury bichlorid and formalin bear a striking resemblance to the finer structure of protoplasm as described by cytologists. These facts, no doubt, have an important bearing upon the various "foam," "reticular," and "pseudo-alveolar" structures of the protoplasm of cells described by Bütschli, Fromann, Arnold, Reinke, and others, and may indicate the effect of fixatives upon colloid solutions, explaining the usual time-worn objections to theories of protoplasmic structure as based upon artificial conditions not present in the normal living cell, and variously interpreted according to the fixative employed.

Studies in the size of colloidal particles have been greatly facilitated by dark-field illumination of the microscopic field or the so-called ultramicroscope devised by Siedentopf and Zsigmondy. Various other methods have been devised for studying the size of colloidal particles, as ultrafiltration by Bechhod²; the weight of a dispersed substance in a given volume by chemical or other analysis; by studies in the density of the dispersed substance and other methods. These studies have indicated that colloidal particles are usually round or at times ovoid, as indicating beginning crystallization; that the main difference between suspensions and colloidal solutions is one of dispersion and not relative size of particles, and that in a true colloidal solution particles of widely differing sizes may be found side by side.

9. *Colloids may be precipitated by electrolytes of opposite sign, as well as by colloids.* In a colloidal solution surface tension constantly tends to make the particles of colloid approach one another, so that the surface may become as small as possible, and in this manner brings about precipitation or coagulation.

In a stable solution this action is counterbalanced by a force of electric repulsion. Pure colloids do not carry an electric charge and are not conveyed by an electric current; their apparent charge depends upon the nature of electrolytes that may be present. Traces of acid and of acid salts give it a positive charge, whereas alkalies and alkaline salts do the opposite (Pauli).

The process of coagulation of proteins, therefore, must depend upon the neutralization of their electric charge, and, as previously stated, this can be accomplished either by electrolytes or by colloids:

(a) Precipitation by electrolytes is best illustrated by the action of a

¹ A good general outline of the subject of colloids may be found in Pauli's *Physical Chemistry in the Service of Medicine*, 1907, translated by Fischer (Chapman and Hall).

² *Ztschr. f. Chem. Ind. Koll.*, 1907, 2, 3.

strong acid on an albuminous solution. The negatively charged particles attract to themselves the positively charged hydrogen ions; their charge is now neutralized, and the force of attraction due to their surface tension is no longer counterbalanced by an electric repulsion. The particles are drawn together, form larger and larger masses, which finally come under the influence of gravity and precipitation takes place.

(b) Precipitation of colloids by colloids is illustrated by the precipitation of albumin by acetic and ferrocyanic acids. The colloid must be of opposite sign. As a result of the acid the particles acquire a positive charge, if they are not so charged already. This charge is then neutralized by the colloidal ferrocyanic acid of negative sign; the surface tension is no longer neutralized by an electric repulsion, and particles come together to form larger masses that are finally deposited as a precipitate.

Instead of precipitating the other, an excess of one colloid may act in a reverse manner. For example, as Neisser and Friedmann have shown, a suspension of particles of mastic in water (made by dropping an alcoholic solution in water) takes on a negative charge, and can be precipitated by positive colloids or ions, such as ferric chlorid. If the dose of ferric chlorid is increased gradually, the precipitate becomes more and more abundant, until an excess of ferric chlorid is present, when the reaction ceases and the precipitate may be redissolved. This has been explained on the assumption that when two colloids of opposite sign are mixed, they tend to fuse and form masses; the addition of an excess of either colloid tends to electrify the masses, causing mutual repulsion and possibly resolution of the masses. Hence the precipitate is soluble in an excess of both substances, just as a precipitate is soluble in an excess either of precipitin or of its antigen.

10. *Absorption* is the taking up of dissolved or volatile substances by finely divided or colloidal bodies. It is a combination between two substances dependent on physical attraction rather than on chemical affinity, and taking place in variable ratios, rather than in simple and constant ones, as occur in a true chemical union. It is believed by many that the two substances entering into the phenomenon of absorption exist as such side by side in the compound, which is to be regarded as an intimate admixture of the two rather than as a new compound.

The lack of definite ratios by which colloids are absorbed has been shown by Bordet in the amount of hemolytic immune body that can be taken up by a given volume of corpuscles—*i. e.*, the amount varies according to whether the corpuscles are added at once or in successive small portions. Thus, in one example, 0.4 c.c. of a hemolytic serum dissolved 0.5 c.c. of corpuscles if added at once; but if 0.2 c.c. of corpuscles was added first and successive amounts of 0.1 c.c. then put in, no solution took place after the one that followed the addition of the first portion. This was explained by Bordet according to the principles of absorption, this observer comparing it with the absorption of a dye by filter-paper. While other explanations are possible, yet exactly analogous phenomena may be seen in the mutual absorption of colloids of opposite sign. Thus, as we have previously stated, the addition of a solution of an electropositive colloid to a solution of an electronegative one tends to repel the particles, with the formation of masses for the purpose of self-protection, and in this manner the process of agglutination and precipitation is begun. But if a small amount of a second colloid is added to the same volume of the others, new aggregates of the two are formed that are less favorable to precipitation and require more of the second colloid to bring about complete precipitation.

ANALOGY BETWEEN THE REACTIONS OF IMMUNITY AND COLLOIDAL CHEMISTRY

With these few brief remarks on the properties and nature of colloids and the close resemblance of cellular protoplasm and fluids to colloids, we may consider briefly the apparent similarity that exists between the colloidal reactions and some of the reactions of immunity. This is especially pertinent for several reasons: it has been shown that cellular protoplasm is colloidal in nature; that antigens are certainly colloidal, and that antibodies, while they may or may not be solutions of colloids, are, in the final analysis, products of cellular activity, and therefore derived from colloidal solutions. Too much emphasis, however, cannot be placed upon the value of interpretation of immunologic phenomena in the test-tube and living animal on the basis of colloidal reactions. As stated by Krogh¹ we must consider colloidal chemical laws in relation to immunologic researches, but our present knowledge of colloidal chemistry is so elemental that only a small part of immunology is explainable by colloidal reactions of alternations and absorptions. A thorough review of the subjects of colloids and catalysis in relation to antigens and antibodies has been recently made by Nicolle and Césari.²

1. **Antitoxins.**—The side-chain theory of Ehrlich was first applied in explanation of the principles of immunity as affording an explanation of the action of toxins, the formation of antitoxin, and the interaction between these. Ehrlich has placed the various phenomena of immunity upon a chemical basis, bringing forward new theories to explain the various discrepancies that were found. For example, it was soon found that both toxin and antitoxin were unstable, and that neutralization of a toxin by the addition of antitoxin was not a simple process, like the neutralization of an acid by an alkali, but, on the contrary, was likely to be exceedingly complicated. This was explained as being due to the degeneration of toxin into various toxoids, which were able to neutralize antitoxin without being in themselves toxic when in a free state. They were likewise found to have a greater affinity for antitoxin than the toxin itself, so that when a toxin was tested and its toxicity determined, it was discovered that more antitoxin was needed to neutralize the mixture than was originally calculated, because the toxoids took no part in testing the toxin, but were active in uniting with antitoxin, and in this manner leaving true toxin unneutralized, and therefore toxic, unless an excess of antitoxin was used.

Analogous conditions may be observed among colloidal solutions. Thus, Danysz has shown that more toxin is neutralized if antitoxin is added at once than when it is added in successive doses. As stated elsewhere, this is explained by Ehrlich upon the assumption that time is allowed for the degeneration of toxin into toxoids to take place, the latter having a greater affinity for the antitoxin. It has been shown, however, that in some cases the addition of a small amount of a second colloid of opposite sign to a colloidal solution may render the solution more stable and protect it from precipitation by an excess of the second substance. Similarly, the amount of colloid necessary to precipitate a constant amount of another colloid is reduced to a minimum if the addition is made at once, and is rendered much greater if the colloid added is made slowly in small amounts, an interval being allowed to elapse after each addition. This is closely analogous to the Danysz reaction, and explains the latter as being due, when antitoxin is added slowly to toxin, to the formation of transitional compounds of toxin

¹ Jour. Infect. Dis., 1916, 19, 452.

² Ann. de l'Inst. Pasteur, 1922, 36, 463.

and antitoxin of diverse nature, requiring more antitoxin for complete neutralization of the toxin than if the antitoxin were added at once and in one dose.

The difference between the L_0 and L_+ dose of a toxin also has an analogy in the reaction of simple colloidal substances. Thus, Bilty has used ferric hydroxid, which neutralizes arsenic trioxid (the antidote for acute arsenical poisoning), and found that the addition of one lethal dose of arsenic to a neutral mixture of the two did not render the mixture toxic, but that several lethal doses were required, just as it is necessary to add several instead of one lethal dose of diphtheria toxin to the L_0 dose.

Thus it would appear that the neutralization of a toxin by an antitoxin has analogies among the known and simple colloidal reactions. One objection to placing the toxin-antitoxin reaction upon a colloidal basis is that both have the same electric charge, *i. e.*, both move toward the cathode, and, as we have seen, for the neutralization and precipitation of colloids the solution of colloids should be of opposite sign. It must be remembered, however, that toxins and antitoxins react in very complex fluids containing other substances consisting of both colloids and electrolytes, and until the electric charge of these in pure form is determined, the apparent similar electric charge of toxin and antitoxin can hardly outweigh the otherwise remarkable analogy it bears to colloidal reactions. The neutralization of toxin by antitoxin is not, however, according to Arrhenius and Krogh,¹ a purely colloidal phenomenon of absorption. Krogh found that toxin absorbed to an organic suspended substance quite as harmful to guinea-pigs as before, the only difference being that it was absorbed more slowly; absorption may explain the union, but does not explain the neutralization.

2. Agglutinins and Precipitins.—Various theories in explanation of the phenomenon of agglutination have been described in a previous chapter. The theory of Bordet appears to be best, and is based upon certain principles of colloidal chemistry. When bacteria are suspended in a fluid free from salt agglutination does not take place because the bacteria carry a similar negative charge of electricity. When, however, ions of positive charge are added, as, *e. g.*, sodium chlorid, the bacteria or other cells are repelled and coalesce to form masses, according to the laws of surface tension, in an effort to protect themselves. Larger masses may be formed that finally come within the influence of gravity and are deposited at the bottom of the test-tube. According to the same laws the addition of agglutinin removes the negative charge of bacteria or other cells, with the consequent formation of clumps and masses. Similar phenomena may be observed in the precipitation of colloidal suspensions of clay in distilled water by the addition of a salt.

Solutions of inorganic colloids, as, for example, that of silicic acid, may agglutinate red corpuscles; bacteria, such as suspensions of typhoid and colon bacilli, may be agglutinated by solutions of the ferric salts.

Just as an excess of one colloid solution will charge masses of the other, resulting in a repelling action and breaking up of the agglutinated clumps, so the addition of an excess of agglutinin is found to prevent agglutination or to give but a slight reaction. This phenomenon has been explained, according to Ehrlich's side-chain theory, as due to the presence of agglutinoids that have a great affinity for the bacteria and unite with them without being active in the free state, owing to a loss of the agglutinophore portion of the molecule. Each cell united with an agglutinoid is one cell less to undergo agglutination by agglutinin, and accordingly in weak dilutions of

¹ Jour. Infect. Dis., 1916, 19, 452.

serum agglutination is feeble or absent, whereas in higher dilutions the phenomenon may be clearly observed.

Other explanations of the action of agglutinins and precipitins, based upon colloidal reactions, have been advanced. Thus Neisser and Friedmann have shown that suspensions of mastic may be "protected" against the precipitating action of ferric hydroxid by the addition of a small amount of organic colloid, such as serum, leech extract, or extract of typhoid bacilli, regardless of whether this colloid is charged positively or negatively or is neutral. The aforementioned observers believe that normal bacteria may be surrounded by a similar protective envelope that prevents the agglutinating action of substances of opposite sign. The action of agglutinin, therefore, would be to remove this layer, so that the ions of opposite electric charge can unite with the bacteria and bring about their agglutination. This may be an explanation of the rôle of salts in the phenomenon of agglutination, the agglutinins removing the protecting envelopes and the salt furnishing the ions of opposite charge that bring about agglutination.

Owing to the fact that a discrepancy arises here for the reason that emulsions of red corpuscles are agglutinated by both positive and negative colloids (ferric hydroxid and cuprum ferrocyanid), Girard, Mangin, and Henri have given the following explanation of agglutination: When a red corpuscle is suspended in a fluid, various salts, especially the sulphates of magnesium and calcium, are diffused, which tends to facilitate the precipitation of negative and positive colloids, so that each corpuscle comes to be surrounded by a layer of precipitated colloid material. This zone of precipitated colloids of either negative or positive charge determines agglutination in the presence of a colloid solution of opposite charge, such as agglutinin or inorganic colloids (silicic acid, etc.).

3. **Hemolysins.**—Reference has been made elsewhere to the original observations of Bordet, showing that red corpuscles may absorb much more hemolytic antibody than is necessary to bring about their lysis, and that this absorption is analogous to colloidal absorption.

Inorganic colloidal solutions, such as that of silicic acid, may produce hemolysis of red blood-corpuscles, *e. g.*, those of the rabbit. Its action is manifested in extremely small doses. It is rendered inert by heat, and gradually deteriorates at room temperature. Furthermore, this inorganic colloid possesses some of the properties of a serum hemolysin; thus mice red corpuscles that have been agglutinated by colloidal silicic acid are dissolved by traces of lecithin or of fresh serum, but not by serum that has been heated to 60° C. (inactivated). An excess of silicic acid tends to prevent hemolysis, which is another example of the action of an excess of one colloidal solution upon another of opposite sign.

Probably saponin hemolysis and the influence of fatty substances, such as lecithin and the fatty acids, upon the phenomenon of hemolysis, are closely related to, or to be explained by, the action of organic colloidal solutions.

The fixation of hemolysin to corpuscles apparently follows the simple partition law rather than being purely a phenomenon of absorption, because the velocity is such as may be readily measured; the colloidal alternation law adequately explains the quantitative features of hemolysis.

COMPLEMENT FIXATION AS A COLLOIDAL REACTION

The intimate relationship of precipitation to complement fixation has suggested that the phenomenon may be a colloidal reaction and principally of absorption. This subject is discussed more fully on page 432.

THE RELATION OF LIPOIDS TO IMMUNITY

It is becoming more and more evident that lipoids bear an important relation to various immunologic processes, especially to certain cytolytic phenomena. This is especially true if the cell walls are composed chiefly of lipoids as regarded by several investigators; these substances would thereby play an important part in protecting the fixed and wandering body cells as well as bacteria.

As the relation of lipoids to various immunologic processes¹ has frequently been described in earlier chapters, as, *e. g.*, where the rôle of lipoids in venom hemolysis, in the Wassermann syphilis reaction, and in the various precipitin reactions in syphilis were considered, a brief résumé may be of service in directing attention to this important and particular phase of immunity.

Lipoids as Antigens.—This important subject has been discussed on pages 147 and 426. It would appear to be the consensus of opinion that pure lipoids are not antigenic and when injected into animals do not produce antibodies. If lipoids are combined with protein, however, the complex may be antigenic and engender the production of various antibodies. This factor probably explains the discrepancies in the reports of various investigators. As previously stated, Jobling and Bull² observed an increase of serum lipase in the sera of rabbits immunized with chicken corpuscles indicated by increased hydrolysis of ethyl butyrate; it may be stated, however, that according to Thiele and Embleton³ hydrolysis of ethyl butyrate cannot be accepted as evidence of lipolytic activity of serum.

Relation of Lipoids to Ferments, Antiferments, and Immunity.—The important investigations of Jobling and Petersen regarding the unsaturated fatty acids of serum being antitryptic have been discussed in the chapter on Ferments and Antiferments. According to these investigators various substances, including the halogens, kaolin, and bacteria, may bring about saturation of these and render the serum proteases active. This may be followed by the digestion of serum or other proteins with the production of toxic substances.

Relation of Lipoids to Opsonins and Phagocytosis.—These have been studied by Walbum,⁴ Stuber,⁵ Arkin,⁶ Dewey and Nuzum.⁷ According to the results observed by Walbum, the administration of cholesterol increases phagocytosis, but Stuber and Arkin found this substance to depress phagocytic activity. This was confirmed by Dewey and Nuzum, who observed that the depressing effect is chiefly on the leukocytes. Graham⁸ has observed that the opsonins are decreased following ether anesthesia, which suggests that these effects may be due to the activity of lipoidal substances. According to Müller⁹ the bacterial lipoids do not increase or decrease phagocytosis and are unimportant in this connection.

The available data bearing upon this subject would indicate, therefore, that certain lipoids in serum, and notably cholesterol, may inhibit phagocytosis, but that the lipoids of the phagocytosed cells are themselves inactive in the process.

¹ Bibliography on Lipoids and Immunity given by Landsteiner, Kolle and Wassermann's Handbuch, 1913, 2, 1240. Also review of literature by Landsteiner, Jahresb. Immunitätsf., 1910, 6, 209.

² Jour. Exper. Med., 1913, 17, 61.

³ Jour. Path. and Bacteriol., 1914, 19, 349.

⁴ Ztschr. f. Immunitätsf., 1910, 7, 544.

⁵ Biochem. Ztschr., 1913, 51, 211; *ibid.*, 1913, 53, 493.

⁶ Jour. Infect. Dis., 1913, 13, 408.

⁷ Jour. Infect. Dis., 1911, 8, 147.

⁸ Jour. Infect. Dis., 1914, 15, 472.

⁹ Ztschr. f. Immunitätsf., 1909, Ref., 1, 61.

Relation of Lipoids to Agglutinins.—Apparently very little investigation has been devoted to this subject. Froin¹ found that corpuscles free of lipoids produce agglutinins, but not hemolysins, when injected into animals. Thiele and Embleton² have likewise found that the lipoids play no part in the production of agglutinins. According to Stuber,³ however, immunization of rabbits with fat-free typhoid bacilli results in diminished agglutinin production. He believes that the agglutinins are produced as a result of the stimulus afforded by the fats liberated after destruction of the bacteria.

This subject requires investigation. At the present time the rôle of lipoids in the production of agglutinins, that is, as agglutininogens, is doubtful, and nothing has been done to show that they are concerned in the mechanism of the agglutination reaction.

Relation of Lipoids to Hemolysis.—(a) From the standpoint of immunity, *venom hemolysis* is of peculiar interest as indicating the possible important relation of lipoids to hemolytic complement. Granting that venom contains a hemolytic amboceptor (Flexner and Noguchi), the complementing substance must be derived from the corpuscles, and, according to Kyes, this complementary agent is represented in lecithin. Kyes was able to produce what he considers are compounds of the hemolysin with lecithin, namely, "lecithids." Whether these "lecithids" are true compounds of hemolysins and corpuscular lecithin or simply the active hemolytic products of the cleavage of lecithin by ferments contained in the venom, is at present unknown. Noguchi and Lieberman have shown that not only lecithin, but soap as well, especially unsaturated fatty acids, and probably protein compounds of soaps and lecithin, may act as the hemolytic complement and activate the hemolysin of the venom. Lipoids from bacteria and trypanosomes have been found to possess similar properties. Hemolytic lipoids have been secured from serum, and the complementary activity of a fresh normal serum may be destroyed by fat solvents, *e. g.*, ether. While other investigators have not been able to confirm Noguchi's attempts to produce an artificial complement of fatty substances of exactly the same properties as serum complement, this work indicates most strongly the close relation of serum complement to lipoids or protein-lipoid compounds.

(b) *The hemotoxic activity of various toxins is probably dependent largely upon their action on the lipoids of red corpuscles.* The saponin substances,⁴ a group closely related to glucosids, and found in at least 46 different families of plants, are strongly hemolytic. Ransom⁵ has found that an ethereal extract of red corpuscles contains a substance that inhibits saponin hemolysis. This substance consists largely of cholesterolin, and it is the presence of cholesterolin in normal serum that inhibits saponin hemolysis. This may be demonstrated experimentally by adding cholesterolin to a solution of a saponin. Noguchi⁶ has shown that lecithin does not possess the same antihemolytic action on saponin. It would appear, therefore, that saponin causes hemolysis by combining with, altering, or dissolving the lipoids of the stroma of corpuscles. The resistance of corpuscles to saponin hemolysis varies in certain diseases, being especially low in jaundice (McNeil⁷).

While saponins, solanins, phallin, and other vegetable poisons are of relatively simple chemical composition and quite unlike proteins, enzymes,

¹ Compt. rend. Soc. de biol., 1912, 72, 154.

² Ztschr. f. Immunitätsf., 1913, 16, 161.

³ Münch. med. Wchn., 1915, 1173.

⁴ Complete literature on saponin, see Kobert, Die Saponinsubstanzen, Stuttgart, 1904.

⁵ Deut. med. Wchn., 1901, 27, 194.

⁶ Univ. of Penna. Med. Bull., 1902, 15, 327.

⁷ Jour. Path. and Bact., 1910, 15, 56.

or toxins, it is possible that bacterial and vegetable hemotoxins, such as tetanolysin, abrin, ricin, croton, and robin, may produce their effects by a similar action on the lipoids of the erythrocytes. Noguchi has shown that cholesterolin inhibits the action of tetanolysin. Landsteiner and Bottori have found that protagon, a brain lipid, possesses the property of binding tetanus toxin, which indicates that this toxin may produce its effects by some action upon the lipoids of nerve-cells.

(c) Whether *serum hemolysins* are lipoidal constituents is doubtful. Jobling and Bull¹ have claimed that immunization of rabbits with hen corpuscles results in an increase of lipase based upon the serum acquiring the property of hydrolyzing ethyl butyrate, but Thiele and Embleton,² claim that the hemolytic power of a serum bears no relationship to its lipolytic power and serum hemolysin is not a lipase.

Relation of Lipoids to the Wassermann Reaction and Precipitation.—*The important relation of lipoids to the Wassermann reaction and certain precipitin or floccule-forming reactions* (Klausner, Porges-Meier, Hermann-Perutz) has been mentioned repeatedly. Just what rôle the lipoids play in these phenomena is not known. While the globulins of syphilitic serums are strongly suspected of being concerned in these processes, their relation is not clear. Klausner³ now believes that the precipitate that forms when distilled water is added to syphilitic serum is due to the high lipid content.

I have previously discussed (page 435) the important relation serum lipoids may bear toward the anticomplementary activity of serum and to non-specific complement fixation by the normal sera of rabbits, dogs, and mules. Removal of these substances by lipid solvents tends to decrease these effects.

Mention has also been previously made (page 429) to the probable rôle of lipoids in the complement-fixation reaction, and more especially to the lipoidal nature of alexofixagens in syphilis and other infections. The syphilis "reagin" is especially important in this connection because it may be dissipated by such lipid solvents as ether and alcohol, while chloroform narcosis is said to render the sera of normal individuals Wassermann positive. Furthermore, the serum lipoids may be increased in syphilis as claimed by Citron and Reicher,⁴ Peritz,⁵ and others, indicating without much doubt that lipoids play an important rôle in the phenomena of serum hemolysis, antihemolysis, non-specific and probably specific complement fixation.

Relation of Lipoids to Anaphylaxis.—This subject is considered in more detail in the chapters devoted to Anaphylaxis. In regard to sensitization it is now well proved that pure lipoids, that is, protein-free lipoids, are not able to act as anaphylactogens. This does not mean, however, that lipoids may not play some part in anaphylactic phenomena. For example, Saula⁶ has observed an increase in soap and fatty acids subsequent to sensitization, and Jobling and Petersen⁷ have noted symptoms and lesions resembling anaphylaxis following the intravenous injection of guinea-pigs with soap. According to these investigators when the antitryptic power of the serum of guinea-pigs is raised (increase of unsaturated fatty acids) or, when soaps are added to the intoxicating dose of protein, the animals are able to resist several times the amount of specific protein fatal for the controls.

¹ Jour. Exper. Med., 1913, 17, 61.

² Jour. Path. and Bact., 1914, 19, 349.

³ Biochem. Ztschr., 1912, 47, 36.

⁴ Berl. klin. Wchn., 1908, 1398.

⁵ Deut. med. Wchn., 1910, 36, 481.

⁶ Compt. rend. Soc. de biol., 1913, 15, 273.

⁷ Jour. Exper. Med., 1914, 20, 468.

TECHNIC OF COLLOIDAL REACTIONS

LANGE'S COLLOIDAL GOLD REACTION

Principles.—According to the exhaustive studies of Zsigmondy¹ on metallic colloids a solution of a protein will precipitate colloidal gold in the absence of an electrolyte. An electrolyte, as sodium chlorid, will in certain concentrations precipitate the colloidal gold itself; if proteins are present this precipitation is inhibited. The degree of protection afforded has been found specific for each protein, and is expressed in terms of milligrams of the protein capable of protecting 5 c.c. of colloidal gold against 0.5 c.c. of a 10 per cent. solution of sodium chlorid.

Lange² endeavored to distinguish between normal and luetic sera by this means on the basis of disturbances in syphilis, but failed; he then sought to measure the protein content of cerebrospinal fluid by the degree of precipitation of gold, but failed again, because he used distilled water as a diluent, thereby throwing the protein, particularly the globulins, out of solution, and rendering them inert. When, however, he used a 0.4 per cent. solution of sodium chlorid as a diluent it was found that the proteins were not precipitated and that this amount of salt was too weak to precipitate the colloidal gold.

It was now possible, according to Lange, to measure the protein content of spinal fluid according to the degree of precipitation, and very interesting and practical results have been secured, although it cannot be stated as proved that the precipitation is due entirely to protein, particularly since it has been shown that the globulins may actually protect colloidal gold against precipitation. Fischer³ has recently, however, disproved the protective value of the globulins and found that fibrinogen plus fibrinoglobulin-euglobulin, and pseudoglobulins possess a flocculating effect upon the colloidal gold solution. Zaloziecki⁴ regards the reaction as a form of immunity reaction; Jaeger and Goldstein⁵ consider it purely physical and probably of an electric nature.

Preparation of Colloidal Gold.—The preparation of colloidal gold is frequently an exceedingly troublesome procedure, and the success of the test depends upon a satisfactory preparation. The method which I shall briefly describe here is after that of Miller, Brush, Hammers, and Felton,⁶ which I have found to yield fairly constant and satisfactory products.

The glassware (beakers, pipets, and test-tubes) must be absolutely clean. They may be washed in hot water with ivory soap; rinsed in tap-water for five minutes; placed in hot bichromate cleaner for half an hour; rinsed in tap- and, finally, triple distilled water. The beakers should be used at once; the pipets and test-tubes are to be dried in a hot-air oven. Thermometers should be cleansed in a similar manner.

It is necessary to use water *triply distilled* in an apparatus *free of rubber connections*. The reagents should be freshly prepared of the best products obtainable.

1. Heat 1000 c.c. of triply distilled water over a good Bunsen burner in a prepared beaker with a thermometer.

¹ Ztschr. f. Anal. Chem., 1901, xl, 697.

² Berl. klin. Wchn., 1912, xlix, 897; Ztschr. f. Chemotherap., 1913, 1, 44.

³ Ztschr. f. d. ges. Exper. Med., 1921, 14, 60.

⁴ Deutsch. Ztschr. f. Nervenhe., 1913, xlvii, 783.

⁵ Ztschr. f. d. ges. Neur. u. Psych., 1913, xvi, 219.

⁶ Bull. Johns Hopkins Hosp., 1915, xxvi, 391.

2. At 60° C. add 10 c.c. of a 1 per cent. solution of Merck's gold chlorid crystals in triply distilled water and 7 c.c. of a 2 per cent. solution of Merck's blue label potassium carbonate in triply distilled water.

3. At 80° C., while stirring briskly, add 10 c.c. of a 1 per cent. solution of Merck's blue label oxalic acid crystals in triply distilled water.

4. At 90° C. remove the burner and, while stirring, add 5 c.c. of a solution of 1 c.c. of Merck's highest purity formaldehyd in 40 c.c. of triply distilled water, or enough to produce an initial pink color.

5. The solution must be neutral in reaction when used, and for this purpose is tested with a 1 per cent. solution of alizarin red in 50 per cent. alcohol. With this indicator the neutral point is a brownish-red tint; an acid solution gives a lemon-yellow, and an alkaline solution a purplish-red color.

To 10 c.c. of the colloidal gold in a clean beaker add 2 drops of indicator. If it is acid, titrate to the neutral point with $n/50$ NaOH; if alkaline, with $n/50$ HCl. Calculate the amount to be added for the amount of colloidal gold solution at hand and neutralize with normal or decinormal solutions of acid or alkali as required.

The following are suitable standards for a good colloidal gold product:

(a) Absolutely transparent and of a brilliant red orange or salmon-red color.

(b) Five c.c. of the solution must be completely precipitated in one hour by 1.7 c.c. of a 1 per cent. solution of sodium chlorid in distilled water.

(c) The solution must be neutral in reaction when used.

(d) The solution must not produce a reaction greater than a No. 1 with normal cerebrospinal fluid, and must give a typical reaction curve with a known paretic fluid.

The Test.—1. Arrange eleven clean, dry test-tubes in a row; put 1.8 c.c. of fresh, sterile 0.4 per cent. NaCl solution into the first tube and 1 c.c. in the following ten.

2. With a clean, dry pipet add 0.2 c.c. of a blood-free cerebrospinal fluid to the first tube and mix; transfer 1 c.c. from the first to the second tube, mix, and proceed in this manner up to and including the tenth tube, from which 1 c.c. is discarded. The eleventh tube is the control and contains no cerebrospinal fluid. The dilutions now range from 1 to 10 to 1 to 5120.

3. Add to each tube 5 c.c. of colloidal gold; mix and stand aside at room temperature over night; the readings are made the next day and recorded by numbers according to the following scheme:

5 = complete precipitation (water clear).

4 = pale blue.

3 = blue.

2 = lilac or purple.

1 = red-blue.

0 = no change.

Types of Reactions.—1. Normal fluid produces no changes at all or, at most, a No. 1 change in the first tube of the series.

2. The typical reaction is observed in general paresis, giving complete precipitation in the first four to eight tubes of the series, with changes of color in most of the remaining ones, as, for example, 5 5 5 5 4 2 1 0 0, and constituting the "paretic curve" of Miller and Levy.¹ A similar curve is usually found in taboparesis. (See following table and Fig. 149.)

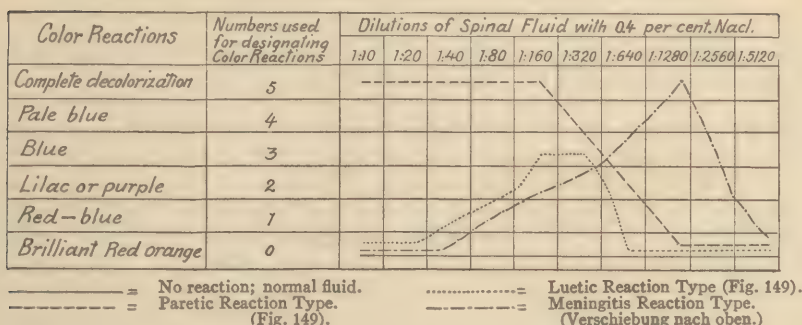
¹ Bull. Johns Hopkins Hosp., 1914, xxv, 123.



FIG. 149.—COLLOIDAL GOLD REACTIONS

The upper set shows a reaction with the cerebrospinal fluid of a parietic ("parietic curve"); the lower set shows a reaction with the cerebrospinal fluid of a tabetic ("fluetic curve").

SHOWING THE FOUR COMMON TYPES OF COLLOIDAL REACTIONS



3. The cerebrospinal fluid in tabes dorsalis usually produces the "luetic zone" curve of a No. 4 intensity, as, for example, 4 4 4 5 5 4 2 0 0 0; precipitation being partial in the first two or three tubes, then becoming complete, and gradually returning to normal through the balance of the series. The changes, however, are not constant or characteristic.

4. The fluids in cerebrospinal syphilis usually yield weak reactions of the "luetic zone" type. In tertiary syphilis without symptoms referable to the central nervous system similar reactions are frequently observed.

5. Fluids from cases of purulent or tuberculous meningitis may give reactions which are usually maximal in the higher dilutions, the "meningitic zone" type and so-called "Verschiebung nach oben." In acute anterior poliomyelitis the cerebrospinal fluid frequently yields reactions similar to the "luetic" and "meningitic" zone types of reactions.

Practical Value of the Colloidal Gold Test.—The reports of a very large number of investigators, including Lange,¹ de Cruris and Frank,² de Cruris and Eberhardt,³ Grulee and Moody,⁴ Eicke, Jaeger⁵ and Goldstein,⁶ Klienberger,⁷ Zaloziecki,⁸ Kaplan,⁹ Miller and Levy,¹⁰ Swalm and Mann,¹¹ Lee and Hinton,¹² Weston, Darling and Newcomb,¹³ Solomon and Welles,¹⁴ Miller, Brush, Hammers and Felton,¹⁵ and others show that under proper conditions the colloidal gold test is highly specific and of value in the diagnosis of general paresis.

A paretic colloidal gold reaction with the cerebrospinal fluid of a luetic person may be the first sign of an incipient paresis.

A paretic reaction with the cerebrospinal fluid of a luetic individual should be regarded as of grave import; while positive symptoms of paresis may not be present, intensive antiluetic treatment may serve to arrest the disease.

The colloidal gold reactions in tabes dorsalis and cerebrospinal syphilis are not characteristic of these diseases, and at most may yield the luetic zone type of reaction, and thereby prove of value in confirming a doubtful diagnosis.

¹ Ztschr. f. Chem. u. v. Gehete., 1913, 1, 44; Berl. klin. Wchn., 1912, xlix, 897.

² Münch. med. Wchn., 1915, lxi, 1209.

⁶ Loc. cit.

³ Münch. med. Wchn., 1914, 61, 1216.

⁷ Arch. f. Psych., 1911, xlviii, 264.

⁴ Amer. Jour. Dis. Child., 1915, ix, 19.

⁸ Loc. cit.

⁵ Münch. med. Wchn., 1913, lx, 2713.

⁹ Ztschr. f. d. ges. Neurol. u. Psych., 1915, xxvii; Jour. Amer. Med. Assoc., 1914, lxii, 511, 246.

¹⁰ Bull. Johns Hopkins Hosp., 1914, 25, 123.

¹² Amer. Jour. Med. Sci., 1914, cxlviii, 33.

¹¹ New York Med. Jour., 1915, xi, 719.

¹³ Amer. Jour. Insan., 1915, lxxi, 773.

¹⁴ Boston Med. and Surg. Jour., 1914, clxxi, 886; *ibid.*, 1915, clxxii, 398.

¹⁵ Bull. Johns Hopkins Hosp., 1915, 26, 391.

THE EPIPHANIN REACTION

Principle.—This reaction is based upon the observation made by Weichardt¹ in 1908; he found that diffusion is accelerated when differently colored solutions of antigen and its specific antibody are brought together. Changes in diffusion are associated with changes in the surface tension, both of which depend on a change in the osmotic pressure. This is the principle made use of by Ascoli in his *miostagmin reaction*, which will be described further on.

Later Weichardt made the reaction more accessible to practical use by introducing into the solution of serums and antigen a system composed of sulphuric acid and barium hydroxid, together with certain catalytic agents. Using phenolphthalein as an indicator, he could show that fresh serums in high dilutions alter the surface tension of the finely divided barium sulphate particles by their colloidal action, so as to increase the absorption of H-ions, thus rendering the solution more alkaline.

This phenomenon has been utilized by Weichardt, under the name of "epiphanin reaction," to determine the occurrence of such interaction of antigen and antibody. The reaction probably depends upon physico-chemical principles of absorption, but the exact nature of the change is not yet understood. The reaction is based upon the following generalizations:

1. Solutions containing colloids—*i. e.*, antigen alone, antiserum alone, or antigen plus non-specific antiserum in certain dilutions—act in the foregoing system by shifting the phenolphthalein end-point (the point of neutralization when acid and alkali are brought together in the presence of this indicator) in the sense of increased OH-ions (pink color).

2. Specific antigens can inhibit the activity of their specific antisera, the specific antigen-antibody combination then becoming evident *in vitro* by a shift of the end-point in the sense of increased H-ion concentration (light color).

Specificity.—The specificity of the reaction has been confirmed by a number of investigators who used the test for the identification of a host of antigen-antibody combinations *in vitro*. The underlying principles have been confirmed by Kraus and Amiradzibi,² Schroein,³ Seifert,⁴ Mosbacher,⁵ and others. The reaction has been applied to a study of various antigens and their antibodies, such as diphtheria toxin, tetanus toxin, typhoid and tubercle bacilli, tumor extracts, and placenta extracts by Weichardt; extracts of syphilitic livers and serums of syphilitic patients by Seifert, Keidel, and Hurwitz.⁶

Technic.—The technic of this reaction has been modified from time to time. The method here given is essentially the latest given by Weichardt,⁷ slightly modified by Keidel and Hurwitz.

Five constituents enter into the test:

1. *The Antigen.*—This is an alcoholic extract of syphilitic liver, prepared in exactly the same manner as for performing the Wassermann reaction. High dilutions of the antigen, ranging from 1 : 100 to 1 : 10,000, are prepared with normal salt solution. As in the Wassermann reaction, not every antigen is satisfactory, a point that can be determined only by making preliminary tests.

2. The patient's *serum* should be fresh, unheated, and highly diluted, the dilutions ranging from 1 : 100 to 1 : 10,000,000. Usually it is better to

¹ Berl. klin. Wchn., 1908, No. 20; Centralbl. f. Bakteriöl., xliii, 143; *ibid.*, xlvii, 39; Ztschr. f. Immunitätsf., 1910, vi, 651; Deutsch. med. Wchn., 1911, No. 4, 154.

² Ztschr. f. Immunitätsf., 1910, vi, 16.

³ Münch. med. Wchn., 1910, 38, 1981.

⁴ Deutsch. med. Wchn., 1910, 50, 2333.

⁵ Deutsch. med. Wchn., 1911, 22, 1021.

⁶ Jour. Amer. Med. Assoc., 1912, lix, 1257.

⁷ Berl. klin. Wchn., 1911, 43, 1935.

use higher than lower dilutions. When too concentrated solutions of serums and of antigen are used, erroneous results are likely to be obtained.

3. A normal solution of sulphuric acid.

4. A saturated solution of barium hydroxid made equivalent to the normal solution of sulphuric acid. In the use of the barium hydroxid it is imperative to prevent its exposure to the air. A solution that has become cloudy, owing to the entrance of carbon dioxid, should not be used. In carrying out the test it is best to pour out the amount of barium hydroxid needed for the test into a rubber-stoppered bottle or test-tube, so as not to contaminate the stock solution.

5. A 1 per cent. alcoholic solution of *phenolphthalein* containing 1 per cent. of a 10 per cent. solution of strontium chlorid. The strontium chlorid has been found to catalyze the reaction.

The Test.—This is conducted as follows: A number of clean beakers of about 50 c.c. capacity are used. For each dilution of the serum a separate beaker is required. One beaker is used for an antigen control, and another to control the system of barium hydroxid and sulphuric acid. Five beakers may be used, Nos. 1, 2, and 3 constituting the main test, No. 4 the antigen control, and No. 5 the system control.

The reagents are added by means of overflow pipets. To each of the first four beakers is added 1 c.c. of the dilute antigen to be used in the test (about 1 : 10,000). To beaker 5 is added 1 c.c. of the salt solution used in making the dilutions of the antigen and the serums. Now 0.1 c.c. of the dilute serum to be tested is added to each of the first three beakers, each beaker, however, containing the same serum in a different dilution. To beaker 5 the same quantity of salt solution is added, but to beaker 4—the antigen control—no serum or salt solution is added.

To each of the five beakers the system of sulphuric acid and barium hydroxid and phenolphthalein is now added carefully. First, 2 c.c. of the normal sulphuric acid solution are added to each; then 2 c.c. of the barium hydroxid, and finally 0.1 c.c. of the phenolphthalein strontium chlorid mixture.

It will be seen that beaker 4—the antigen control—contains all the constituents of the test-beakers 1 to 3 except serum. To make beaker 4 qualitatively as well as quantitatively equal to beakers 1 to 3, 0.1 c.c. of the dilute serum (the average of the dilutions of serum which are used in the test) is now added to beaker 4, the reaction having already taken place.

The addition of the sulphuric acid and barium hydroxid requires great care. Since the reaction depends on small differences in acidity or alkalinity, it is obvious that slight errors will vitiate the results. For the acid and the alkali separate pipets are used. After emptying the pipet of its content of acid or alkali, the last traces adhering to the inside of the pipet are removed by washing. These washings are later added to the beakers to which they belong. In filling the pipets with acid or alkali, the latter should first be drawn up into the pipet at least once, and then emptied again before the pipet is finally filled for delivery into the next test-beaker. Only by careful attention to these points in the technic can reliable results be obtained.

Reading the Results.—If beakers 1 to 3 contained the antiserum to the antigen used, a positive epiphanin reaction will be obtained, and if the barium hydroxid and sulphuric acid were previously carefully adjusted to each other, it will be found that beakers 1 to 3 will be lighter than the antigen control, beaker 4. The presence of a specific antigen-antibody combination has shifted the phenolphthalein end-point in the sense of increased

H-ion concentration. The exact differences in the alkalinity between beakers 1 to 3 and beaker 4 can be quantitatively determined by titration with $n/100$ sulphuric acid, and the results expressed as a curve.

If the antigen and antibody were not specific, the epiphanin reaction will be negative. Beakers 1 to 3 will be more alkaline than the antigen control, beaker 4, because, as previously pointed out, serums alone or antigen with non-specific serums shift the phenolphthalein end-point in the sense of increased OH-ion concentration.

The results may be plotted as curves. The titration values in $n/100$ sulphuric acid are placed on the ordinates, and the serum dilutions on the abscissæ. The positive values are plotted above the line and the negative values below the line. No reaction is regarded as positive unless it gives a titration value of at least 0.05 c.c. $n/100$ sulphuric acid. Values below 0.05 c.c. are easily within the limits of error.

The following method, employed by *Seifert*, is much simpler, but is open to the error on account of using the antigen and serum in too concentrated a state. In a small test-tube place 0.1 c.c. of a 1 : 10 solution of the serum in normal salt solution, and add 0.1 c.c. of an alcoholic extract of syphilitic liver. To this slowly add 1 c.c. of decinormal sulphuric acid and 1 c.c. of a solution of barium hydroxid of the exact concentration needed to neutralize the sulphuric acid solution. On the addition of the drop of the phenolphthalein solution the fluid turns red when the serum is from a syphilitic, whereas no change in tint occurs with non-syphilitic serum.

Practical Value.—The reaction appears to be of considerable value in the diagnosis of *syphilis*. With serums and antigen in proper dilutions, the results closely parallel those secured by the Wassermann reaction. Keidel and Kurwitz report positive reactions with luetic serums in about 75 per cent. of their cases. The reaction was found highly specific in that syphilitic extracts gave negative reactions with serums of non-syphilitic persons and patients suffering from malignant disease. Extracts of normal fetal liver and beef heart gave negative reactions with serums of syphilitic persons.

Positive reactions have also been found in *malignant disease*, as with the antigens of carcinoma and sarcoma. Keidel and Hurwitz obtained 16 positive reactions in a series of 24 serums of persons suffering with definite or suspected malignant disease. Burmeister did not find the reaction of value in cancer.

The epiphanin reaction has also been used in the diagnosis of pregnancy, but sufficient work has not been done to render an expression as to its merits of value at this time.

THE MIOSTAGMIN REACTION

Among the very large number of immunity reactions employed in attempts to secure a diagnostic test for cancer, the "miostagmin reaction" of Ascoli and Izar¹ is the only one thus far devised that claims the serious attention of the clinician.

Principles.—This reaction is founded on the fact, noted by Ascoli, that by the mixing of an antigen and its corresponding antibody there results a reduction of the surface tension of the liquid containing these, which may be demonstrated by counting the number of drops of the fluid in a given volume (usually 1 c.c.), under constant conditions. Normal serum diluted with salt solution is first tested, and the number of drops found in a cubic centimeter determined with a specially devised instrument known as Traube's

¹ Münch. med. Wchn., 1910, 57, 62, 182, 403.

stalagmometer. The antigen is so diluted that when mixed with this normal serum it does not increase the number of drops more than 1 in a cubic centimeter. When properly diluted patient's serum and antigen are mixed it may be found that the number of drops is increased from 2 to 8 in a cubic centimeter. This constitutes a positive reaction. The reaction is apparently due to the lowering of surface tension, so that more and smaller drops are found; hence the term "miostagmin" has been applied to the test, the word being derived from the Greek, meaning "small drop."

The reaction is said to be sharply specific and very delicate, so that antigens diluted up to 1 : 100,000,000 or higher may be detected. The technic requires considerable practice and experience or erroneous results are quite likely to occur.

The exact nature of the reaction is not known. The antigens are soluble in alcohol, but their nature is obscure. The antibody involved in the reaction is referred to as the miostagmin, but its relation to other antibodies is also unknown. It is probably a physicochemical or colloidal reaction, and for this reason it has been placed in this chapter.

Technic.—The *antigen* is most difficult to prepare. A recent method described by Ascoli is as follows:

1. Cut non-degenerated portions of malignant tumor (cancer or sarcoma) into small pieces and dry *in vacuo* or spread out in a thin layer on clean glass plates and keep at a temperature of 37° C.

2. Pulverize the dried substance and extract with pure methyl alcohol (in the proportion of 5 gm. to 25 c.c.) for twenty-four hours at 50° C. in closed vessels, and shake occasionally.

3. Filter while still hot, and allow the filtrate to cool, and then filter again through Schleicher and Schull's filter-paper No. 590.

4. It is now necessary to *titrate the antigen* and to determine in what dilution it should be employed. Various dilutions of the antigen are made with distilled water, as, e. g., 1 : 10, 1 : 25, 1 : 50, 1 : 100, 1 : 150, 1 : 200, etc. A fresh normal serum is diluted 1 : 20 with normal salt solution, and 9 c.c. of this are mixed with 1 c.c. of the various antigen dilution. Into another tube place 9 c.c. of the diluted serum and 1 c.c. of distilled water. *All test-tubes, pipets, and other glassware used must be perfectly dry.*

The tubes are gently shaken and placed in an incubator at 37° C. for two hours. The drop number for each fluid is then estimated by Traube's stalagmometer. This instrument is merely a finely and elaborately graduated pipet with a central bulbous reservoir. The dropping end of the instrument ends in a flattened ground base, thus insuring uniformity in the size of the drops. The instrument is so graduated that a fraction of a drop can be estimated. *That antigen is to be chosen that does not alter the drop number for normal serum by more than 1 drop in a cubic centimeter—the strongest dilution that fulfils this condition being chosen.*

The Test.—The patient's serum is diluted 1 : 20 with normal salt solution and its drop number determined. Then take two tubes, and into one place 9 c.c. of diluted serum plus 1 c.c. of antigen dilution; into the other place 9 c.c. of diluted serum plus 1 c.c. of distilled water. A third tube may be prepared, which should contain 9 c.c. of normal serum (1 : 20) plus 1 c.c. of the same antigen dilution. A fourth tube contains 9 c.c. of a known positive serum (1 : 20) from a case of cancer and 1 c.c. of the antigen dilution.

All tubes should be carefully labeled, their drop numbers determined, and then placed in an incubator at 37° C. for two hours or in the water-bath at 50° C. for one hour. At the end of this time they are removed, allowed to cool, and the drop number of each is determined.

The controls are first examined to show that the antigen has not undergone any change. *Variations of the number above 1.5 or 2 drops (as compared with the control containing distilled water instead of antigen) are regarded as positive reactions.* The increase in drops is seldom greater than 8.

MIOSTAGMIN REACTION IN CANCER

9 C.C. SERUM (1 : 20) PLUS 1 C.C. ANTIGEN (1 : 200).	DROPS PER C.C. AFTER INCUBATION AT 37° C. FOR TWO HOURS.	DROPS PER C.C. CONTROLS 9 C.C. SERUM (1 : 20) PLUS 1 C.C. DISTILLED WATER AFTER INCUBATION.	RESULTS.
Normal serum	56.0	56.4	Negative.
Known cancer serum	62.4	59.2	Positive.
Unknown serum for diagnosis	61.2	60.0	Positive.
Unknown serum for diagnosis	57.2	57.0	Negative.
Unknown serum for diagnosis	62.4	59.8	Positive.

Other Methods for Preparing Antigens.—Various methods for preparing antigen and conducting the test are to be found in the literature, and it is extremely difficult to arrive at a correct conclusion as to which is the best method for preparing antigen. Among these methods for the preparation of antigen other than those previously described are the following:

1. After securing the alcoholic extract described elsewhere evaporate it to dryness. Again extract in methyl alcohol and evaporate. Extract with warm ether, renewed several times during the course of twenty-four hours. Dry, and repeat the extraction several times until the alcohol remains colorless. Evaporate the alcoholic and ethereal extracts at 50° and 37° C. respectively. A yellowish-red, sticky mass results. Dissolve this in a large amount of water-free ether. Filter, and evaporate at room temperature until a slight powdery precipitate is deposited. This solution constitutes the stock antigen, which, however, may require still further concentration.

2. A synthetic *cancer* antigen may be prepared by grinding up 0.5 gm. of lecithin (ovolecithin Merck, or lecithin Richter), and extract it with 50 c.c. of acetone for twenty-four hours at 50° C. Filter through Schleicher and Schull's filter-paper No. 590 until clear. Just before it is to be used it should be diluted with water in such amount that 1 c.c. will contain the largest amount that does not cause a marked reduction of surface tension in normal serum. As a rule, this dilution is between 1 : 50 and 1 : 100 (Köhler and Luger¹).

3. A *syphilis* antigen may be prepared by extracting 0.5 gm. of dried and powdered syphilitic liver with 50 c.c. of absolute alcohol for two hours at 37° C. with frequent shaking. Filter, and concentrate to 10 c.c.

4. A *bacterial* antigen, as *e. g.*, one of typhoid bacilli, may be prepared as follows: Wash off five forty-eight-hour agar cultures of typhoid bacilli with 5 c.c. of normal salt solution for each tube. Cover the emulsion with toluol, and shake vigorously for several hours. Place in an incubator at 37° C. for forty-eight hours, and filter through a sterile Berkefeld filter. This filtrate may be used as antigen, or it may be used in preparing an alcoholic extract in the following manner: To the original aqueous filtrate add 50 c.c. of absolute alcohol. Allow the mixture to stand for one-half hour, shake,

¹ Wien. klin. Wchn., 1912, 25, 1114.

centrifugate, and then mix the sediment with 20 c.c. of absolute alcohol. Shake thoroughly once more, and again centrifugate. Combine the two extracts, and concentrate on the water-bath to about 20 c.c.

Practical Value.—This test is quite delicate, and errors due to faulty technic are quite likely to creep in. Unless all precautions are rigidly observed, the results are worthless. Although an extensive literature has accumulated upon this test, the method has not come into general use and it is not possible to express an opinion of its value in the study of disease.

Ascoli and Izar especially have advocated the test in the diagnosis of cancer. In 100 cases of malignant tumors they obtained 93 positive reactions; in 103 cases of other diseases they obtained only one positive reaction. Tedesko, Stabilini, Leitch, Kelling, and others have reported favorably upon the practical value of the test in the diagnosis of cancer, a good review of the literature up to 1911 being given by Bernstein and Simons.¹ Burmeister² has found that a negative reaction has some value in excluding cancer, and is of more value in arriving at a diagnosis than a positive reaction, *i. e.*, it has a higher negative than a positive value. Gouwens,³ however, has recently reported that the reaction failed with *Bacillus paratyphosus* immune rabbit-serum of high titer regardless of the methods employed for preparing antigen and conducting the tests.

The test has also been used in the diagnosis of typhoid fever, paratyphoid fever, syphilis, tuberculosis (positive only in active cases), echinococcus disease, etc. Obviously, other methods of diagnosis, such as the agglutination reaction and the Wassermann reaction, have superseded this test in practical diagnosis. The method possesses, however, considerable theoretic interest and is worthy of further investigation.

¹ Amer. Jour. Med. Sci., 1911, 142, 852.

² Jour. Infect. Dis., 1913, 12, 459.

³ Jour. Infect. Dis., 1922, 31, 237.

CHAPTER XXVIII

ALLERGY. ANAPHYLAXIS AND HYPERSENSITIVENESS

It is generally believed that when an animal previously injected with an antigenic substance is subsequently reinjected with the same substance, the antibodies induced by the first injection are reinforced, and that a continuation of the process of immunization will eventually lead to a high degree of immunity. Under certain circumstances, however, this is not the case, because severe and even fatal symptoms, as well as other manifestations, may set in after the second injection, indicating that, instead of being immune, the animal is indeed hypersusceptible or hypersensitive to the affects of the antigenic substance.

Before experimental investigation of this subject was undertaken not a few observations were made and described by the early workers in the fields of bacteriology and immunity that correspond exactly with the phenomenon of *anaphylaxis*, as we understand it today, although the true explanation of their unexpected results was not suspected, and they were modestly ascribed to faulty technic, embolism, toxicity of the inoculum, etc. A typical example is clearly recorded by Flexner¹ in 1894: "Animals that had withstood one dose of dog-serum would succumb to a second dose given after the lapse of some days or weeks, even when this dose was sublethal for a control animal." Rabbits were being employed and the reactions were perfect examples of anaphylaxis in these animals to dog-serum. From this it followed that the discovery that the experimental injection of such ordinary innocuous substances as normal serum and milk may produce violent symptoms and death gave rise to much surprise and incredulity, since scientists had long been accustomed to regard the reaction of an animal to an injection as a process of immunization, or diminished sensitiveness, instead of one of increased sensitiveness. Here, as Besredka remarked, the rules of immunity are "standing on their heads."

In this chapter will be presented the known facts regarding allergy and the theories that have been advanced in explanation of its nature and mechanism, the consideration of allergy in its practical application to medicine being left for the following chapter.

Historic.—The first observation of allergy as it occurs in an infectious disease was probably made by Jenner in 1798. This investigator observed the sudden appearance of an "efflorescence of a palish red color" about the parts where variolous matter had been injected into a woman who had had cowpox thirty-one years before.

In 1839 Magendi found that rabbits that had been injected with egg-albumen died after a repetition of the injection, a phenomenon strikingly similar to that observed sixty-five years later by Theobald Smith following injections of horse-serum. This phenomenon was subsequently studied thoroughly by Rosenau and Anderson and Otto.

While the effects of diphtheria and tetanus antitoxins were being studied, peculiar and apparently paradoxical results were occasionally observed during immunization of animals with the bacterial toxins. Thus in 1895 Brieger² reported the case of a goat that was highly immunized against tetanus and

¹ Medical News, 1894, 65, 116.

² Ztschr. f. Hyg., 1895, 101.

yet was subject to tetanus. In 1901 von Behring and Kitashima¹ reported similar findings with diphtheria in horse immunized against that infection. At this time it was shown that the results could not be due to the cumulative effect of the toxin, and the explanation offered aimed to show that the process was purely histogenetic, and based upon the assumption that receptors attached to the body cells had a closer affinity for toxin than the free (antitoxin) receptors in the blood-stream. At the present time toxin hypersusceptibility is held by some to be a true anaphylactic reaction brought about by protein substances in the toxin filtrate; others regard von Behring's explanation as satisfactory. Further reference to this subject will be made later on in this chapter.

Richet's Studies.—The fundamental observations upon which our present knowledge of anaphylaxis is based were made in 1898 by Hericourt and Richet.² These observers found that repeated injections of eel-serum into dogs gave rise to an increased susceptibility to this substance, instead of immunizing the dogs against the serum. These studies were continued by Richet and Portier³ with extracts of the tentacles of certain sea anemones. These studies showed that a second injection of the poison into dogs, given after an interval of several days, is followed by greater and more intense activity than marked the first injection. If the animal survives, however, the disease is conquered more readily after the second than after the first injection. As previously stated, Richet coined the word "anaphylaxis," meaning "without protection," and indicating that the first injection destroyed any natural resistance that the animal might possess against the poison (actionocongestin). From these studies he concluded that two different substances are contained in eel-serum and in the tentacles of actinians, one concerned in establishing an immunity, and the other in calling forth a hypersensitiveness; thus far, however, the separate existence of these two hypothetic substances has not been proved.

Arthus Phenomenon.—In 1903 Arthus,⁴ at the instigation of Richet, showed that similar results may be obtained with non-toxic substances, like serum and milk. On injecting rabbits at definite intervals with normal horse-serum, he found that the first two or three doses were absorbed, whereas subsequent injections, given subcutaneously, led to increasingly severe local reactions (Arthus phenomenon). If the animals, however, were first injected subcutaneously and later intravenously, or intraperitoneally, serious symptoms of dyspnea, convulsions, and diarrhea, and even death resulted. At this time the specificity of anaphylaxis was discovered; as stated by Arthus: "the rabbit sensitized by and for serum is not so for milk, and vice versa."

Von Pirquet's Early Studies.—For a long time urticarial eruptions were occasionally observed to follow transfusion of blood from lambs and other lower animals to persons suffering from anemia and similar conditions. Soon after diphtheria antitoxin was discovered the medical profession was shocked to learn of the sudden death of the healthy child of an eminent German professor following a prophylactic injection of the serum. In 1902 von Pirquet began the study of these clinical manifestations with a child in Escherich's clinic who, after receiving a second dose of horse-

¹ Berl. klin. Wchn., 1901, xxxviii, 157.

² Compt. rend. Soc. de biol., 1898, 53.

³ Compt. rend. Soc. de biol., 1902, liv, 170; 1903, lv, 246; 1904, lvi, 302; 1905, lviii, 112; 1907, lxii, 358, 643; 1909, lxvi, 763; 1909, lxvi, 810; 1909, lxvi, 1005. Ann. de l'Inst. Pasteur, 1907, xxi, 497; 1908, xxii, 465.

⁴ Compt. rend. Soc. de biol., 1903, lv, 817; 1906, lx, 1143. Archiv. internat. de physiol., 1908-09, vii, 472.

serum ten days after the first, on the same day developed symptoms of fever and a rash. On the basis of this observation von Pirquet¹ reached the conclusion that the prevailing views regarding the length of the incubation period of an infectious disease could not be correct. He therefore propounded the theory that the organism concerned in the etiology of disease calls forth symptoms only when it has been altered by antibodies, the period of incubation representing the interval necessary for the formation of these antibodies.

In conjunction with Schick, von Pirquet endeavored to study all infectious diseases from the same point of view, especially smallpox, measles, recurrent fever, streptococcus infections, and the reactions to cowpox virus, tuberculin, and mallein. Later these same observers² studied the symptoms following injection and reinjection of horse-serum, designating the train of symptoms *serum sickness*. They emphasized that a *single* injection of serum may suffice to bring about the symptoms, and that this immediate reactivity possesses diagnostic value in so far as it enables us to decide whether a previous infection has occurred. How near the astute Jenner came to reaching the same conclusion is shown in the following abstract from his report in 1798:

"It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces the smallpox. *Indeed, it becomes almost a criterion by which we can determine whether the infection will be received or not* (italics ours). It seems as if a change, which endures through life, had been produced in the action, or disposition to action, in the vessels of the skin; and it is remarkable, too, that whether this change has been effected by the smallpox or the cowpox, that the disposition to sudden cuticular inflammation is the same on the application of variolous matter."

Von Pirquet at this time proposed the term "allergy," from *ergeia*, reactivity, and *allos*, altered, meaning altered energy or a changed reactivity, as a clinical conception expressing a truth without binding any one to a theory based upon bacteriologic, pathologic, or biologic findings.

Theobald Smith Phenomenon.—While von Pirquet was making these studies, great impetus was given the experimental study of anaphylaxis by the observation of Theobald Smith, who found that guinea-pigs that were used for standardizing the strength of diphtheria antitoxin after a second injection of serum frequently presented symptoms of a serious character, such as great restlessness, dyspnea, itching of the skin, and violent convulsive seizures. In fully 50 per cent. of the animals death occurred within half an hour.

Simultaneously Rosenau and Anderson³ in this country and Otto⁴ in Germany undertook the study of this phenomenon. The first-named investigators showed most conclusively, by a thorough series of experiments, the action of horse-serum and other substances in guinea-pigs, and proved that serum sickness was due to some constituent of the serum independent

¹ Zur Theorie der Infektionskrankheiten (Vorläufige Mitteilung), April 2, 1903. Zur Theorie der Vakination, Verhandl. d. Gesellsch. f. Kinderh., Kassel, 1903.

² Wien. klin. Wchn., 1903, xvi, 758, 1244; 1905, xviii, 531. Die Serumkrankheit, Leipsic, Deuticke, 1905; Münch. med. Wchn., 1906, liii, 66. For a full bibliography on this subject of allergy up to 1910 see von Pirquet, Archiv. Int. Med., 1911, vii, 259, 383.

³ Bull. 29, Hyg. Lab., U. S. P. H. and M. H. S., 1906; Bull. 36, Hyg. Lab., April, 1907; Jour. Infect. Dis., 1907, iv, 552; Bull. 45, Hyg. Lab., June, 1908; Bull. 50, Hyg. Lab., 1909; Jour. Amer. Med. Assoc., 1906, xlvii, 1007; Archiv. Int. Med., 1909, iii, 519.

⁴ von Leuthold Gedenkschrift, 1905, i; Münch. med. Wchn., 1907, liv, 1665; Kolle and Wassermann, 1908, ii, 255.

of the antitoxic antibodies, as normal horse-serum yielded exactly similar results.

Among the earlier studies of anaphylaxis of importance were those of Weichardt.¹ These were made with extracts of placental cells, and later with the proteins of pollen, in relation to hay-fever. Wolff-Eisner² wrote a treatise that had as its fundamental idea the belief that hypersensibility was due to endotoxins liberated by a lysin formed as a result of the first injection. Also among the earliest and most valuable studies upon the nature of anaphylaxis, and showing the important relation of proteins to the process, are those of Vaughan³ and his co-workers; indeed, the studies of Smith, Rosenau and Anderson, Vaughan and Wheeler, Gay and Southard, Auer and Lewis, and others have gained for America a prominent part in the development of this important subject.

Practical Importance of Hypersensitiveness.—Since these original discoveries were made a large amount of clinical and laboratory research has been devoted to a study of hypersensitiveness in man and the lower animals. Developed along with the subject of serum therapy it was but natural that most of the earlier work should have dealt with the sensitization of guinea-pigs with horse-serum, and curiously enough this combination has since proved the most typical example of anaphylaxis.

A natural result of these discoveries was a closer study of hypersensitiveness in man so that the tuberculin reaction, food and drug idiosyncrasies were soon placed in the category of anaphylactic phenomena. Not a few physicians know in a general way of the fatal character of serum anaphylaxis in the guinea-pig and a fear of eliciting similar reactions in man by the administration of sera for prophylactic and curative purposes is wide-spread among the profession and shared by many of the laity.

Few subjects in immunology are as important and as perplexing as hypersensitiveness. Despite a very large amount of investigation we are still ignorant of much that is fundamental, and the introduction of numerous theories and new terms and the tendency for drawing conclusions on the basis of analogy with observations on the lower animals has tended to confuse the subject as applied to man. It is extraordinary how the phenomena and lesions of hypersensitiveness vary in different species of animals, and the subject in a broad and general way is one of great importance in man in relation to disease and immunity, serum, vaccine and chemotherapy, and various idiosyncrasies to foods, drugs, and other substances.

Classification of Phenomena.—As previously stated, the word "anaphylaxis" was coined to express a condition of absence of protection and the antithesis of prophylaxis and immunity. It has since been widely adopted for designating all conditions of hypersensitiveness in man and the lower animals.

Typical anaphylaxis, as occurring in guinea-pigs sensitized with horse-serum, is known to be caused by protein antigens interacting with specific antibodies which may be passively transferred by injecting the serum of a sensitive animal into a normal animal. Coca⁴ has recently advocated that the word "anaphylaxis" be reserved for phenomena of this kind and that "allergy" be used for designating all similar phenomena which have not been clearly and definitely proved to be antigen-antibody reactions.

¹ Berl. klin. Wchn., 1903, No. 1.

² Ztschr. f. Bakteriöl., 1904; Berl. klin. Wchn., 1904, xli, 1105, 1131, 1156, 1273; Münch. med. Wchn., 1906, liii, 217.

³ Summarized in Protein Split Products, Lea & Febiger, 1913.

⁴ Tice's Practice of Medicine, W. F. Prior Company, New York, 1920, 110.

Coca's classification is, therefore, as follows:

Hypersensitiveness.	
Anaphylaxis.	Allergy.

In my opinion Coca's insistence upon the demonstration of antibodies in the serum of a sensitized animal by passive transfer is too rigid; antibodies may be developed in a sensitized animal, including man, without being successfully transferred by injecting the serum into another animal. Throughout the study of hypersensitiveness in man and the lower animals it would appear that "*altered reactivity*" of the cells of certain organs and tissues expressed as an exaggerated susceptibility to the exciting substance stands out as the predominating and constant departure from the normal, and for this reason we agree with Doerr on the suitability of adopting von Pirquet's term *allergy* for designating hypersusceptible states. There is apparently so much difference in the nature of the exciting causes of allergy with such substances as serum, milk, and egg white at one end and drugs, as quinin and mercury, at the other, that in the interests of orderly classification the subject of allergy may be divided according to the nature of the exciting causes. In our opinion it is doubtful whether we are justified in making the division on the basis of *being able to demonstrate* antibody production by the exciting substances. Our technic for discovering antibodies is too imperfect for warranting such a classification; it is possible that antibodies may be present in cells when their presence in the blood may not be demonstrable.¹

The proposed classification is, therefore, as follows:

Allergy.	
A. Hypersensitiveness { Natural Acquired (Exciting agents are non-protein substances).	B. Anaphylaxis { Natural Acquired (Exciting agents are proteins or protein derivatives).

Definitions.—**Allergy** may be defined as a condition of unusual or exaggerated specific susceptibility to a substance which is harmless in similar amounts for the majority of members of the same species.

The exciting substance may or may not be primarily a protein and may or may not engender the production of *free* antibodies demonstrable in the serum. The lesions and symptoms are uniform in any one species of animal for different exciting substances, but different from those produced in animals of another species and different from the normal or physiologic effects of the substances.

Anaphylaxis may be defined as a state of allergy characterized by "unusual or exaggerated susceptibility of the organism to foreign proteins" (Rosenau). In my classification I have made the term embrace protein derivative products. For example, Koch's old tuberculin may be a proteose and we regard the tuberculin reaction as an anaphylactic phenomenon.

Natural anaphylaxis designates a condition in which the time of sensitization is unknown; practically it covers those cases of immediate reaction to injections of horse-serum in children or adults who have never received previous injections. It also includes those cases of food intolerance encountered in children.

Acquired anaphylaxis designates the usual type in which sensitization is known to have been produced by the administration of a foreign protein.

¹ Handbuch. d. path. org. Kolle-Wassermann, 2d ed., 112, 947.

It embraces the majority of anaphylactic states; however, the *time* of sensitization may not be discoverable in anaphylaxis to pollens, bacteria, foods, and various effluvia of the lower animals.

Hypersensitiveness may be defined as a state of allergy characterized by unusual or exaggerated susceptibility to non-protein substances as drugs. Wolf-Eisner has advanced the theory that these substances (crystalloids) may combine with plasma proteins with the production of an altered protein sufficiently foreign to prove antigenic. If this is ultimately shown to be true despite the negative evidence at present, or, if it is shown that drugs excite hypersensitiveness by union with cells with the production of *foreign intracellular antigens*, drug idiosyncrasies or hypersensitiveness may be classified as anaphylactic.

Natural hypersensitiveness designates those cases of intolerance to drugs exhibited by individuals who have never before taken the drug. Not infrequently it remains undiscovered until adult age.

Acquired hypersensitiveness designates those cases of intolerance to a drug developing during the course of its administration; apparently one type of the arsphenamin reaction is attributable to acquired hypersensitiveness.

Terminology.—The substance producing the allergic state has been designated *anaphylactogen* by Richet; *sensibiligen* or *sensibilisinogen* by Besredka, and *allergen* by von Pirquet and Schick. The term “anaphylactogen” is most commonly employed but, in our opinion, should be reserved for designating the exciting agents of anaphylaxis. The best general term for embracing all states of hypersusceptibility is one carrying the idea of “sensitizing” the body cells; for this purpose *sensitino-gen* appears expressive.

In the artificial production of allergy the first injection of sensitino-gen is called the *sensitizing dose* and the process is commonly known as *sensitization*.

The antibody regarded as responsible for the allergic reaction has been called *toxogen* by Richet, *sensibilisin* by Besredka, *sensitizin* by Weil, *reaction body* by Otto, *albuminolysin* by Nicolle, and *allergen* by von Pirquet. The term most frequently employed is *anaphylactin*, but I am uncertain regarding who coined this word.

The dose of sensitino-gen (anaphylactogen), bringing on the allergic reaction, is commonly spoken of as the intoxicating dose. In the typical guinea-pig-horse-serum anaphylactic reaction Besredka designates the first injection of serum as the “sensitizing injection” and the second injection ten to twelve days later as the “toxic or exciting injection.”

SYMPTOMS AND PATHOLOGY OF ALLERGY

In the lower animals the allergic reactions which have received most study have been those of anaphylaxis induced by the injection of foreign sera, egg-albumen, and vegetable proteins in guinea-pigs, rabbits, and dogs.

The pathologic changes and symptoms of anaphylaxis vary considerably in different animals, but in any species are the same for different anaphylactogens regardless how widely separated in biologic origin or chemical characters the latter may be. While the same may be true in man this has not been proved and for the obvious reason that our data is accumulating more slowly.

Man.—In man the symptoms and pathologic changes of allergy are quite varied. Acute fatal anaphylaxis following the administration of horse-serum is fortunately rare and such accidents have usually occurred among individuals subject to “horse asthma” and sensitive to horse proteins to an extreme degree. The usual form of anaphylaxis in man is either an im-

mediate syncopal attack following the intravenous injection of serum or the development of what has been called "serum sickness" characterized by urticarial rashes, joint pains, adenitis, and high fever.

Allergy to the effluvia of the lower animals, as the dandruff of horses and cats, and to pollens is usually expressed by coryza, sneezing, lacrimation, and "asthma." Likewise allergy to bacterial proteins derived from infections of the respiratory tract are generally expressed as a form of "asthma."

Allergy to foods and drugs may be manifest by acute symptoms of swelling of the lips and tongue, vomiting, diarrhea, and violent intestinal pain; chronic allergy to foods is generally expressed by various skin eruptions, of which urticaria and eczema are prominent.

With many of these sensitinogens, including the products of certain micro-organisms (tuberculin, mallein, etc.), local allergic reactions may be produced by application to abrasions of the skin, intracutaneous and subcutaneous injection.

In all of the allergic reactions of man the main lesion is vasomotor paralysis characterized by dilatation of blood-vessels and serous exudation.

With this brief statement we shall pass to a consideration of anaphylaxis in the lower animals, experimentation having given us some insight into the mechanism of the process. Allergy in man and the relation it bears to immunity and disease will be discussed again in the following chapter.

Guinea-pig.—This animal gives the most constant and the most intense symptoms. According to Doerr, guinea-pigs are four hundred times as sensitive an anaphylactic reagent as the rabbit.

Horse-serum, when injected into normal guinea-pigs, gives rise to no symptoms. As much as 20 c.c. may be injected into the peritoneal cavity, and small amounts may even be injected into the brain, without causing any untoward symptoms.

When a small dose of serum is injected intravenously, intraperitoneally, or subcutaneously, and ten days later a second injection is made, the animal develops symptoms of acute anaphylactic asphyxia, which, in the majority of instances, terminates fatally. "In five or ten minutes after injection the pig becomes restless and then manifests indications of respiratory embarrassment by scratching at the mouth, coughing, and sometimes of spasmodic, rapid, or irregular breathing; the pig becomes agitated, and there is a discharge of urine and feces. This stage of exhilaration is soon followed by one of paresis or complete paralysis, with arrest of breathing. The pig is unable to stand, or if it attempts to move, falls upon its side; when taken up it is limp; spasmodic, jerky, and convulsive movements now supervene. This chain of symptoms is very characteristic, although they do not always follow in the order given. Pigs in the state of complete paralysis may fully recover, but usually convulsions appear, and are almost invariably a forerunner of death. Symptoms appear about ten minutes after the injection has been given; occasionally in pigs not very susceptible they are delayed thirty to forty-five minutes. Animals developing late symptoms are not very susceptible and do not die. Death usually occurs within an hour, and frequently in less than thirty minutes. If the second injection be made directly into the brain or circulation, the symptoms are manifested with explosive violence, the animal frequently dying within two or three minutes" (Rosenau.)

Anaphylaxis in the guinea-pig may be prolonged, milder, and finally disappear, leaving the animal in an apparently normal state. These symptoms embrace coughing or sneezing, discharge of urine and feces, restlessness, erection of the hair, and scratching. Sometimes acute symptoms develop

ing immediately after an injection are followed by depression, characterized by somnolence, in which the reflexes are maintained.

H. Pfeiffer¹ has shown that a depression of the temperature is a constant finding in the severer forms of anaphylaxis in the guinea-pig. In fatal cases this decrease may be as much as from 7° to 13° C. Some relation exists between the extent and the duration of the fall of temperature and the severity of the symptoms. During acute anaphylaxis the blood shows a leukopenia—a diminution in complement—and, as shown by Friedberger,² a delay in or a loss of coagulability. The most striking change observed after death is permanent distention of the lungs, resembling emphysema, described by Gay and Southard, and particularly by Auer and Lewis.³ The lungs do not collapse, but remain fully distended, forming a cast of the



FIG. 150.—SECTION OF ANAPHYLACTIC LUNG OF GUINEA-PIG SHOWING EMPHYSEMA; ALSO INFOLDING OF BRONCHIAL MUCOSA.

pleural cavities. The alveoli are distended and, in some instances, the walls may be ruptured (Fig. 150). The walls of the secondary and tertiary bronchi are contracted, with infoldings of the normally thick mucosa, due to contraction of the smooth muscle by peripheral action, death really resulting from inspiratory immobilization of the lungs. The heart continues to beat long after respiration has ceased, and as shown by the electrocardiographic studies of Königsfeld and Oppenheimer,⁴ the disturbances of this organ are accounted for as occurring in consequence to asphyxia. Rosenau⁵ and Gay and Southard⁶ have also described minute hemorrhages in various organs and mucous membranes.

¹ Wien. klin. Wchn., 1909, 14, 989, 1227, 1375.

² Ztschr. f. Immunitätsf., l'orig., 1909-10, 8, 636.

³ Jour. Exp. Med., 1910, xii, 172.

⁴ Ztschr. f. d. ges. exper. med., 1922, 28, 106.

⁵ Bull. No. 32 of the Hyg. Lab., 1906.

⁶ Jour. Med. Research, 1908, xix, 1, 5, 17.

In non-fatal or protracted anaphylactic shock of the guinea-pig the lungs are less inflated than in acute shock. Auer, however, has described partial inflation and it is not improbable that the symptoms are caused by slow asphyxia instead of by liver changes as suggested by Dale. A marked fall in body temperature is characteristic and has been advocated by Pfeiffer and others as a quantitative criterion of the degree of shock, particularly in animals that present few other symptoms and ultimately recover. According to Friedberger and Mita¹ the injection of smaller amounts of antigen may result in an elevation of temperature, which is an important observation in relation to bacterial anaphylaxis in infection.

Rabbit.—Reference has been made elsewhere to the pioneer work of Arthus, who first described the local anaphylactic reaction about the site of subcutaneous injection. He also described objectively the most important symptoms of acute anaphylactic death in the rabbit, as well as the more ordinary type, which ends in recovery.

In acute and fatal anaphylactic shock in the rabbit Auer² found slow respiration, weak or absent heart action, general prostration, the sudden

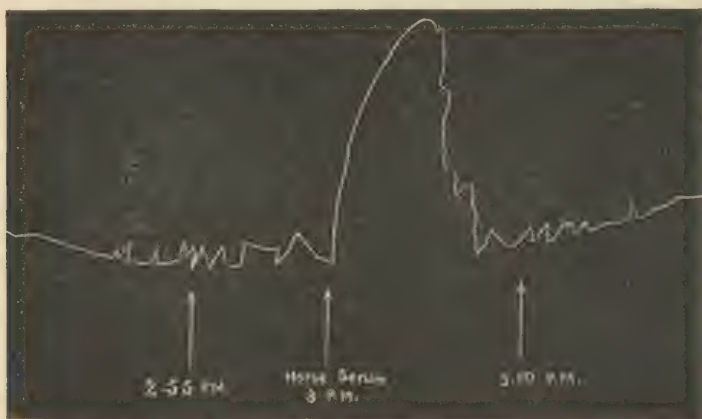


FIG 151.—ANAPHYLACTIC CONTRACTION OF EXCISED SENSITIZED GUINEA-PIG UTERUS (SCHULTZ-DALE METHOD).

falling of the animal on its side, a short clonic convulsion, increased peristalsis, and expulsion in feces and urine. Immediately after the injection the animal remains quiet for a brief period and then suddenly begins to run about violently and aimlessly, when it falls over with the head thrown backward and still making running movements. Or this period of initial excitement may be lacking and its place taken by a somnolent state interrupted by muscular twitchings. Finally the animal succumbs with a few short gasps and with the eyes in exophthalmos. The blood pressure rises at first to be followed by gradual reduction as the heart beat becomes progressively slower. Löewit found the initial rise to be of central origin, as it is lacking when the spinal cord has been severed. In protracted or mild anaphylaxis the animal presents an increased rate of respiration, muscular weakness, and after a few days progressive loss of weight. Death is ascribed to a vascular or cardiac shock or to a failure of the heart action of peripheral origin, mostly affecting the right side, and due to a form of chemical rigor. The muscle may be gray, stiff, very tough to the finger-nail, and non-

¹ Ztschr. f. Immunitätsf., 1911, 10, 216.

² Jour. Exp. Med., 1911, xiv, 476.

irritable. Further evidence of the importance of heart failure in anaphylaxis in the rabbit is furnished by the electrocardiographic study of Auer and Robinson.¹ Blood coagulability is delayed.

Anaphylactic shock of the rabbit is, therefore, much different from the symptoms and lesions presented by the guinea-pig. Instead of the lungs being inflated they are usually collapsed. Coca² has recently shown that there is marked obstruction to the pulmonary circulation apparently due to tetanic contraction of the muscular coat of the pulmonary arterioles. It is possible that a similar vascular constriction of the vessels in the neighborhood of a subcutaneous injection of serum following the initial dilation, may be responsible for the necrosis of the Arthur reaction. The liver may participate in the anaphylactic reaction of the rabbit, being usually the seat of congestion, as shown by Weil.³

Cats.—Anaphylaxis in the cat has been studied especially by Schultz,⁴ who observed that cardiac disturbances followed. Horse-serum, however, was found markedly toxic in effect, even in the unsensitized animal, as little as 0.25 c.c. per kilo killing young, normal cats, and 0.1 c.c. per kilo causing a fall in blood-pressure both in the normal and in the sensitized animals.

Dogs.—In these animals the most constant symptom of anaphylaxis is an initial and transitory rise in blood-pressure, followed by a prompt fall of from 80 to 100 mm. of mercury. This was first described by Biedl and Kraus,⁵ and subsequently by Eisenbrey and Pearce,⁶ Robinson and Auer.⁷ The general symptoms are not as violent as are those that occur in the guinea-pig and death is infrequent. Following intravenous injection of the intoxicating dose of serum there may be great restlessness, marked prostration, and vomiting, tenesmus, and involuntary discharge of feces and urine. If death does not occur, a condition of hemorrhagic inflammation in both the large and the small intestine may develop, called by Richet "chronic anaphylaxis," and by Schittenhelm and Weichardt, "enteritis anaphylactica."⁸ Robinson and Auer, by an electrocardiographic study, detected cardiac changes consisting of disturbance of the heart impulses, abnormalities in ventricular contractions, and other interferences with the mechanism of the heart, due probably to the effect of horse-serum on the peripheral cardiac tissue, and independent of the drop in blood-pressure or any effect upon the central nervous system. The heart changes do not appear to exert a primary influence on the blood-pressure, which is due to an effect upon the splanchnics, and is probably a secondary factor in anaphylaxis or vascular shock of the dog. In many instances there is leukopenia, with loss of mononuclear cells. Coagulation of the blood is delayed, a condition first described by Biedl and Kraus,⁸ who believed it to be due, probably, to a decrease in thromboplastin or an excess of antithrombin. Pepper and Krumbharr⁹ have shown that, by adding small amounts of thromboplastin to the non-coagulating, post-anaphylactic, oxalated plasma, the coagulability of the blood will be restored.

As shown by Manwaring¹⁰ and confirmed by the studies of Voegtlin and Bernheim,¹¹ Deneke,¹² and others, the liver plays an important rôle in ana-

¹ Jour. Exp. Med., 1913, xviii, 450.

² Proc. Soc. Biol. and Med., 1919, 16, 47

³ Jour. Immunology, 1917, 2, 525.

⁴ Jour. Phar. and Exper. Therap., 1911-12, 3, 302.

⁵ Wien. klin. Wchn., 1909, xxii, 365.

⁶ Jour. Pharmacol. and Exper. Therap., 1912, iv, 27.

⁷ Jour. Exper. Med., 1913, xviii, 556.

⁸ Jour. Infect. Dis., 1914, xiv, 476.

⁹ Wien. klin. Wchn., 1909, ii, 363.

¹⁰ Ztschr. f. Immunitätsf., 1910, 8, 1.

¹¹ Jour. Pharm. and Exper. Therap., 1911, 2, 507.

¹² Ztschr. f. Immunitätsf., 1914, 20, 501.

phylaxis in the dog. These investigators have shown that if the liver is excluded by Eck fistula or other means anaphylaxis does not develop. The predominant change is congestion; Weil¹ has calculated that the liver may contain as much as 60 per cent. of the blood during shock. Pearce and Eisenbrey² also noted congestion of the liver and abdominal veins and doubtless these liver changes are primarily responsible for the reduction in blood-pressure and decreased coagulability of the blood.

Mice and Rats.—White mice and rats, while they may not develop acute anaphylactic asphyxia, such as is observed in guinea-pigs, do react to horse-serum, as was shown by Schultz and Jordan. This reaction is evidenced by restlessness, marked irritability of the skin, involuntary passage of urine and feces, and temperature and blood-pressure changes.

Horses; Sheep; Cattle; Hogs.—Hoskins³ has described the following symptoms produced in these animals by intravenous injection:

Horses show uneasiness, an anxious expression, dyspnea, and trembling of certain groups of muscles, sweating, colicky pains and retching, tenesmus, frequent defecation and micturition, and nasal discharge.

Sheep show rapid and labored respiration and cyanosis, lacrimation, nasal discharge and frothing, protrusion of the tongue and swallowing movements, muscular weakness, trembling, and collapse.

Hogs show similar symptoms of restlessness, muscular weakness, and collapse, dyspnea and cyanosis, vomiting, micturition, and defecation.

Cattle usually show immediate trembling, muscular weakness, and collapse if the injection is continued; lacrimation, dribbling, and frequent micturition and defecation.

The pathologic changes do not appear to have been studied with sufficient detail.

THE PATHOLOGIC PHYSIOLOGY OF ALLERGY

Whereas the lesions and symptoms of anaphylactic shock here described in different species of animals are those commonly observed with serum proteins, they vary in no essential when any protein agent is used when the conditions of dosage and administration are the same. It is evident, however, that no one symptom, or group of symptoms, can be regarded as characteristic of anaphylaxis in all animals. The various species present widely differing pictures with the same protein substance, and these differences are best explained on the ground of changes in the anatomic structure and physiologic reaction of different animals. Thus, Schultz has shown that serum anaphylaxis is essentially a matter of hypersensitization of smooth muscle in general, and that, during anaphylactic shock, all smooth muscle contracts. In the guinea-pig this effect is most evident in the bronchi, owing to the peculiar, though normal, anatomic structure of the mucosa, which is relatively thick as compared with the lumen, so that contraction of the smooth muscle throws it into folds that completely occlude the bronchi, causing death from inspiratory asphyxia. The bronchial mucosa of dogs, rabbits, and rats, however, is relatively thin and poor in smooth muscle tissue, which may account for an entire absence of transitory respiratory difficulties during anaphylactic shock in these animals. In the dog the most marked effect is apparent upon the smooth muscle of the gastrointestinal tract, contraction resulting in setting up vigorous intestinal peristalsis, vomiting, and involuntary emptying of the urinary bladder.

¹ Jour. Immunology, 1917, 2, 542.

² Jour. Infect. Dis., 1910, 7, 565.

³ Vet. Alumni Quarterly (Ohio University), 1919, 7, No. 1.

The characteristic initial rise in blood-pressure may be due to constriction of the splanchnic, pulmonary, coronary, and systemic arteries, followed by a condition of paresis and a fall in blood-pressure. The cardiac muscle is also involved, particularly on the right side, as shown by Robinson and Auer, and this favors a venous accumulation of blood. In the rabbit a similar effect is noted upon the smooth muscle of the blood-vessels, and particularly on the heart, as well as upon the gastro-intestinal tract. Our present knowledge would ascribe these effects, therefore, to a local or peripheral action of the protein upon smooth muscle, and not primarily on the central nervous tissues, as was originally believed.

The fall in blood-pressure, therefore, appears to be a most constant and primary factor. In guinea-pig the heart continues to beat after respiration ceases, but this phenomenon may be due to mechanical and other factors dependent upon the extreme pulmonary emphysema. Fall in blood-pressure and congestion of the splanchnic area may produce cerebral anemia, and be responsible in some measure for the respiratory disturbances, the retching, the involuntary expulsion of urine and feces, the great depression and muscular weakness, and the speedy recovery when death does not result.

In man the marked urticarial and other rashes and the inspiratory asthma of those peculiarly sensitive to a protein due to a narrowing of the bronchi, the latter being analogous to the condition observed in the guinea-pig, the diarrhea, and the secondary drop in blood-pressure, all indicate a similar action on smooth muscle. This also provides an adequate pharmacologic explanation of the action of atropin, sedatives, and anesthetics in alleviating or masking the symptoms of acute anaphylaxis.

Aside from the severe fall in blood-pressure and temperature, other effects of anaphylaxis are leukopenia, local and general eosinophilia (Vaughan,¹ Moschowitz,² Schlecht and Schwenket³), and reduced coagulability of the blood. Pfeiffer⁴ found poisonous substances in the urine during anaphylactic intoxication, and Hirschfeld⁵ detected a pressor substance in the serum of intoxicated guinea-pigs.

The single prominent feature of anaphylaxis in all species of animals is the effect upon non-striated muscle. The lesions and symptoms in animals of different species are readily explained upon differences in the distribution of involuntary muscle. The exciting substance, whatever it may be, but commonly designated as "anaphylatoxin," produces immediate and transitory or protracted effects due to contraction which may be followed by relaxation. Such structural changes in the cells of various organs as have been described are readily explainable on the basis of vascular occlusion or dilatation with transudation.

It may well be that tissues other than non-striated muscle are involved; changes in the involuntary muscles have received particular attention, because they are so readily detected and graphically recorded by Dale's method. As will be discussed in the mechanism of allergy nothing is known of what actually occurs in the cell during the allergic reaction.

Apparently some changes occur in the antigen participating in the anaphylactic reaction, as shown by Manwaring and Crowe,⁶ who observed a loss of toxicity of antigen diffused through the livers of sensitized animals. Apparently there is an increase of amino-acids resulting from proteolysis in the tissues but, if this occurs at all, it is not large enough to be of sig-

¹ Ztschr. f. Immunitätsf., 1911, 9, 458.

² New York Med. Jour., January 7, 1911.

³ Arch. exp. Path. u. Pharm., 1912, 68, 163.

⁴ Ztschr. f. Immunitätsf., 1911, 10, 550.

⁵ Ztschr. f. Immunitätsf., 1912, 14, 466.

⁶ Jour. Immunology, 1917, 2, 517.

nificance. For example, Auer and Van Slyke¹ were unable to demonstrate an increase of free aminonitrogen in the lungs of anaphylactized guinea-pigs and Barger and Dale² failed to find an increase of non-coagulable nitrogen in the liver. However, in the blood of anaphylactized guinea-pigs there may be an increase in non-coagulable and urea nitrogen, but it is not known whether this comes from the tissues or from the antigen-antibody reaction in the blood.

ANAPHYLACTOID PHENOMENA

Criteria of True Anaphylaxis.—In allergy due to protein substances (anaphylaxis) the following criteria should be capable of demonstration:

1. The toxicity of the antigen must depend upon previous sensitization; that is, the substance must not produce similar symptoms in non-sensitized animals of the same species.
2. The symptoms and lesions must be those characteristic of anaphylaxis for the species of animal under study.
3. After recovery from shock there should be exhibited some degree of desensitization under proper conditions.
4. The possibility of the symptoms being caused by thrombosis or embolism must be excluded.

The demonstration of passive sensitization cannot be insisted upon.

Anaphylactoid Reactions.—The literature upon anaphylaxis abounds with descriptions of the reactions produced in guinea-pigs, rabbits, dogs, and other animals, following the intravenous injection of serum that has been acted upon by such substances as agar, inulin, kaolin, starch, etc. These investigations were devoted to the production *in vitro* of poisons believed by many to be responsible for anaphylactic shock. These substances may be protein in character, decomposition products of proteins, or non-protein colloids. The injection of these may result in embolism or thrombosis with serious circulatory and respiratory disturbances. Man-wareing and Crowe have observed embolic lesions in the lungs and designated the reaction as "pseudo-anaphylaxis."

The subject has been extensively studied by Karsner and Hanzlik³ with thirty colloidal agents and a variety of methods, including intravenous injection, histologic studies of perfused organs, protection by atropin and epinephrin, as well as test-tube studies on hemolysis and hemagglutination. Many of the agents caused serious circulatory and respiratory disturbances, but both clinically and pathologically were generally different from true anaphylaxis. These investigators regard anaphylactoid phenomena as of a colloidal nature and refer to them as "colloid shock." Peptone was found to produce symptoms and lesions more nearly like true anaphylaxis, but there is a greater tendency for thrombosis, hemorrhage, and edema of the lungs. The toxicity of the protein decomposition products was found quite similar to that of histamin and the effects produced by these substances together with the primary toxic action of beef- and eel-sera for guinea-pigs have been classed as anaphylactoid.

SENSITINOGENS (ALLERGENS)

Sensitinoogens or allergens are substances capable of exciting or inducing a condition of specific hypersensitiveness or allergy. It is a general term and embraces both the protein and non-protein substances.

¹ Jour. Exper. Med., 1913, 18, 210.

² Biochem. Jour., 1914, 8, 670.

³ Jour. Pharmacol. and Exper. Therap., 1919, 14, 229, 379, 425, 449, 463, 479.

Kinds of Sensitinoogens or Allergens.—A large number of substances are known to excite allergy or hypersusceptibility in man; the list for the lower animals is much smaller because our knowledge of allergy occurring among them is practically confined to anaphylaxis induced by the injection of such foreign substances as horse-serum, milk, egg-albumen, bacteria, and vegetable proteins. Some of the lower animals suffer from hypersensitiveness to certain foods and even to drugs, but these subjects do not appear to have received much attention. The immediate death of healthy and unused laboratory animals (rabbits, dogs, and guinea-pigs), sometimes following the first injection of a foreign serum in subtoxic amounts, suggests that examples of natural anaphylaxis among them may occur, although such incidents are usually designated as anaphylactoid and ascribed to embolism and thrombosis.

According to the proposed classification of allergy the exciting substances are divided into those known to be proteins and those generally regarded as non-proteins. The former is the larger group, responsible for anaphylaxis to proteins of both animal and plant origin and are designated as *anaphylactogens*. This term is most generally employed. The latter embrace the allergic principles of certain plants commonly regarded as glucosids and responsible for hypersensitiveness to poison ivy, oak, sumac, and other plants; also certain drugs, as the alkaloids of quinin bark, opium, belladonna leaves, and the like, and some of the elements, as mercury, iron, arsenic, and other substances, which will be discussed later.

PROTEIN SENSITINOGENS (ANAPHYLACTOGENS)

The group of proteins known to produce anaphylaxis in man is very large and derived from both animal and vegetable sources.

Practically any foreign soluble protein will produce sensitization and intoxication of susceptible animals. Bacterial substances, extracts of plant tissues, purified vegetable proteins, and proteins derived from invertebrates and cold-blooded vertebrates have all been found capable of acting as anaphylactogens when introduced in a soluble and unaltered condition into an animal.

The proteins concerned must be foreign to the circulating blood of the injected animal, but they may be tissue proteins of the same animal—*e. g.*, syncytial cells—that are not normally present in the blood. Indeed, Uhlenhuth and Haendel¹ claimed to have sensitized a guinea-pig with the dissolved lens of one eye so that it reacted to a subsequent injection of the lens of the other eye. Proteins in solution are more active than those in suspension or in partial solution, and in general tissue proteins are less active than proteins in the blood, lymph, and secretions, but even keratins may produce anaphylaxis when dissolved (Krusins²); Uhlenhuth³ has obtained positive results with proteins from mummies. As previously stated, the altered protein of an animal may be reinjected again into the animal and induce an anaphylactic reaction. Recently Richet⁴ has directed attention to this phenomenon, which he calls *indirect anaphylaxis*, through observing an intense leukocytosis in a dog which reached the maximum on the eighth day following a second chloroformization.

In many cases the means or mechanism of sensitization is unknown; in

¹ Ztschr. f. Immunitätsf., 1910, 4, 761.

² Arch. f. Augenheilk., Suppl., 1910, 47, 47.

³ Ztschr. f. Immunitätsf., 1910, 4, 774.

⁴ Quoted in Jour. Amer. Med. Assoc., 1914, lxii, 711.

some instances the hypersusceptible state appears to be inherited, but the majority are apparently acquired. As previously stated, instances of natural anaphylaxis in the lower animals to these proteins are almost unknown, but some of them have been employed for inducing anaphylaxis experimentally in guinea-pigs, rabbits, and dogs:

Zoösensitinogens (anaphylactogens)	<ol style="list-style-type: none"> 1. Alien sera and corpuscles. 2. Certain foods, as eggs, milk, and meats. 3. Effluvia as dandruff, and expired organic matter of alien species. 4. Products of animal parasites.
Phytosensitinogens (anaphylactogens)	<ol style="list-style-type: none"> 1. Foods of vegetables and plant origin. 2. Pollens (hay-fever). 3. Products of bacteria and fungi.

Serum Anaphylactogens.—Since the pioneer discoveries in anaphylaxis were made with horse-serum injected into guinea-pigs and rabbits followed up and stimulated by the development of serum therapy in the prophylaxis and treatment of disease, it was but natural that most of our knowledge of anaphylaxis has been developed by investigations employing serum. Curiously enough the combination of horse-serum and guinea-pigs employed by Rosenau and Anderson, Otto, and others in a study of "Theobald Smith's phenomenon" has since proved the ideal anaphylactogen and test animal for eliciting the typical anaphylactic reaction.

Serum of course is a mixture of different simple proteins capable of acting as anaphylactogens. These will be discussed shortly in a consideration of the chemistry of these sensitinogens. Practically the sera of all warm and cold-blooded animals have been found capable of acting as anaphylactogens when introduced in a soluble and unaltered condition into animals of different species. Not infrequently, however, the immediate reactions sometimes observed following the first intravenous injection of serum are anaphylactoid rather than anaphylactic, due to toxicity of the sera or to embolism and thrombosis.

Food Anaphylactogens.—Allergy of some persons to various foods of animal and plant origin has been known for many years as "idiosyncrasies." Apparently it has been observed only in human beings, but probably may occur among the lower animals as well. The proteins of these foods are regarded as the anaphylactogenic substances although in fruits (strawberry) the exciting agent may be a glucosid and lacking in antigenic activity. A large variety of foods are now known in this connection, notably buckwheat, eggs, milk (casein), meats, strawberries, etc. The mechanism of sensitization is not definitely known although experiments by Wells and others, discussed under Active Sensitization, indicate that absorption may take place from the intestinal canal. The lesions and symptoms produced are frequently immediate and striking and referable to the gastro-intestinal and cutaneous system; on the other hand, the manifestations may be chronic and the subject will be discussed in greater detail in the following chapter.

Hair and Other Zoö-anaphylactogens.—The acute coryza and asthmatic-like attacks of some persons brought on by inspiration of the effluvia from the skins, hair, and expired air of the horse, cat, dog, rabbit, guinea-pig, and other domestic animals, including the feathers of the goose and other fowls, are regarded as anaphylactic reactions excited by the proteins contained in these substances. Here again the phenomenon appears to be encountered only among human beings; at least there does not appear to be instances recorded as occurring among the lower animals.

Pollen Anaphylactogens.—Hay-fever of both the spring and autumnal

types is commonly regarded as allergic reactions to the proteins of pollens. Direct contact with the plant is not necessary for producing a reaction, the pollens carried in air currents being sufficient when inspired. Why some persons are allergic and the majority are not, and why the allergy appears to be confined to the human race are unknown; doubtless a fundamental cause is inherited in some instances at least and Coca believes that this is always the case.

In addition to the pollens some persons are susceptible to principles contained in the leaves of such plants as poison ivy, poison oak, poison sumac, and other plants. The lesions are almost solely cutaneous and the exact chemical nature of the exciting substances is unknown. At present they are regarded as glucosids and for this reason I have classed them among the non-protein sensitinoogens, but it is possible that traces of protein may be present and constitute the sensitinogen.

Bacterial and Protozoan Anaphylactogens.—Sensitization of both man and the lower animals may be brought about by the proteins of various bacteria and protozoa, either during infection or by means of active sensitization with vaccines. The best known examples in this connection are sensitizations to tubercle, typhoid, glanders, and contagious abortion bacilli, the gonococcus, pneumococcus and meningococcus, *Treponema pallidum* and *T. echinococcus*.

Sensitization in bacterial infections appears to be of two kinds. One is apparently due to poisons belonging to the proteoses produced only by the living bacterium in the tissues and doubtfully in culture-media; tuberculin is the best known example. According to Kraus and others tuberculin hypersensitiveness is found in man and the lower animals only in the presence of active tuberculosis. It is doubtful whether this kind of allergy can be induced by injections of Koch's old tuberculin; the exciting agent or anaphylactogen is produced only in the tissues as a secretory product of the bacillus or as a derived protein due to action of bacterial poisons upon the cellular or plasma proteins in the focus of infection.

The second variety of anaphylaxis to bacteria and protozoa appears to be due to the proteins (nucleoproteins) of the microparasites which may be prepared from artificial cultures. Instances of this kind of anaphylaxis are not lacking; they are encountered during active immunization with vaccines and are apparently responsible for a type of bronchial asthma due to sensitization by bacteria upon the mucosa of the upper and lower respiratory tract. Probably the earliest clinical observation on anaphylaxis was that by Jenner, who noted the occurrence of skin reactions following cowpox vaccination among those immune and anaphylactic to the smallpox virus; Rosenau and Anderson likewise conducted some of their earliest experiments with the proteins of typhoid, colon, and other bacilli, being among the first to see the relation between serum and bacterial anaphylaxis.

Toxin Anaphylactogens.—This brings up the interesting question as to whether toxins are anaphylactogens, a subject previously mentioned in the historic review of this subject. Instances of hypersensitiveness to diphtheria and tetanus toxins were early observed in attempts to immunize horses in the production of antitoxins. As it is extremely difficult, if not impossible, to isolate a toxin free from other constituents of the medium into which it was excreted by micro-organisms, this question cannot be answered in a definite manner. There is no direct proof, however, that toxins sensitize, although the protein in the toxin filtrate may serve to do so. In 1902 Vaughan and Gelston¹ showed that the poison contained in the cellu-

¹ Trans. Assoc. Amer. Phys., 1902, 17, 308.

lar substance of the diphtheria bacillus is an entirely different one from the toxin elaborated by the same micro-organism, results that were confirmed in 1911 by Friedberger and Reiter,¹ working with the dysentery bacillus. Thus *hypersensitiveness to toxins is probably not an anaphylactic phenomenon*, but is due to a greater affinity of the body cells for the toxin. This explains the so-called *paradox of Kretz*, who found that while the injection of an accurately neutralized toxin-antitoxin mixture produces no bad results in a normal animal, in one that has been previously actively immunized with toxin the reverse occurs. Apparently the sessile receptors have a stronger affinity for toxin than have the free receptors, and, accordingly, the toxin becomes dissociated and combines with the cells.

This view is also substantiated by the observation that the symptoms of intoxication caused by the toxin used for immunization are not those of anaphylaxis, which, for a certain animal, are the same regardless of the source of the protein. Toxin hypersensitiveness does not seem to be transmissible to normal animals, whereas in anaphylaxis the condition may be transmitted (passive anaphylaxis).

Whether or not *endotoxins* act as anaphylactogens cannot be definitely stated. If they do, their action and effects are intimately connected with those ascribed to the protein contained in the bacterial cell. It is unlikely, however, that they play any rôle in inducing hypersusceptibility, as their toxicity is usually apparent soon after injection, and before sensitization has occurred.

Physical State of Anaphylactogens.—The results of experiments all tend to support the theory that proteins in solution are most powerful in producing anaphylaxis, because they are able to come into intimate contact with body cells, and cell permeation is probably necessary for the most complete sensitization. This explains in part the conflicting statements concerning the effect of heat on the sensitizing properties of blood-serum. Rosenau and Anderson found that animals could not be sensitized with serum that has been heated at 100° C., whereas Doerr and Russ placed the point at 80° C. Besredka showed that the sensitizing properties are in part at least dependent upon the physical condition of the protein, and that heating undiluted blood-serum coagulates the protein and leads to a decrease of its anaphylactogenic properties. Similarly, Vaughna found that proteins that were insoluble in water, as, for example, edestin, sensitize more readily when dissolved in salt solution. The same factors are operative with the protein used for intoxication, the physical state of the protein substance having a direct bearing on the rapidity with which shock is produced. Temperatures high enough to disrupt and destroy proteins are, however, equally destructive to their sensitizing properties.

There are, however, a few proteins that are not made insoluble by heat, and these, except gelatin, have been found by Wells to be antigenic despite boiling; examples are casein,² ovomucoid,³ so-called proteoses of seeds,⁴ and beta-nucleoproteins.⁵

Rosenau and Amos⁶ have demonstrated that proteins in a volatile state, as in the exhaled breath of men, when condensed and injected into guinea-pigs will sensitize these animals to subsequent injections of human

¹ Ztschr. f. Immunitätsf., 1911, 11, 493.

² Jour. Infect. Dis., 1908, 5, 449.

³ Ibid., 1909, 6, 596; 1911, 9, 147.

⁴ Ibid., 1915, 17, 259.

⁵ Ztschr. f. Immunitätsf., 1913, 19, 599.

⁶ Jour. Med. Research, 1911, xxv, 35.

serum. While it is doubtful if the complex molecule possesses the power of passing into the air in a gaseous form, it may probably exist in colloidal solution. Rosenau was also able, by keeping guinea-pigs in stables together with horses, to sensitize them to horse-serum. These experiments are of fundamental importance in explaining instances of human anaphylactic phenomena among those sensitive to horse protein, as, *e. g.*, persons seized with sneezing and asthma when they come near horses—and also tend to show how minute may be the quantity of protein capable of sensitizing and intoxicating body cells.

Chemistry of Protein Anaphylactogens.—The purest known proteins act as anaphylactogens or sensitizers; in fact, the purer the protein, the more thoroughly it sensitizes the animal and the smaller is the dose necessary to produce intoxication. The crystallized proteins of hemoglobin, egg-albumen, and such pure vegetable proteins as edestin and excelsin are powerful sensitizers. According to Wells,¹ nothing less than an entire protein molecule will suffice to produce anaphylaxis, although Zunz² claims to have observed typical reactions with the proteoses of fibrin, and Abderhalden³ obtained one with a synthetic polypeptid. It is not necessary, however, for a protein, in order to be active, to contain all the known amino-acids of proteins, for certain vegetable proteins, *e. g.*, hordein and gliadin, which lack one or more amino-acids, such as glyocoll or tryptophane, may produce typical reactions. Presumably, the inability of pseudoproteins, such as gelatin, to act as anaphylactogens depends upon their deficiency in aromatic radicals.

Wells has obtained negative results with purified nucleoproteins, as well as with the isolated components of nucleins, such as histon and nucleic acid.

The Structural Basis of Anaphylactogenic Activity and Specificity.—While, therefore, it is probable, although it has not been definitely proved, that nothing less than the entire protein molecule is capable of producing the typical reaction, the questions arise whether the whole molecule, or only a certain group thereof, determines the specificity, and whether the whole molecule, or only a portion, is concerned as the sensitizing agent. It is now generally accepted that both the sensitizing and the intoxicating agents are one and the same protein, and the older view, which held that in a mixed protein substance, such as blood-serum, corpuscles, egg-albumen, etc., one protein is present that sensitizes and another that intoxicates, is probably erroneous. Besredka, for instance, finds that when a protein used to produce intoxication is heated it is less likely to prove fatal, and he concludes that proteins contain a thermostabile sensitizing and a thermolabile intoxicating portion. Doerr and Russ, however, have shown by carefully conducted experiments, that heat affects both properties of proteins to the same degree. Since pure proteins, as, *e. g.*, highly purified edestin, which is believed to be a chemical unit, act as exquisite sensitizers and intoxicants, it seems reasonable to believe that the sensitizing and poisonous group are constituents of the same protein substance. Whether or not both sensitizing and intoxicating groups are contained in each single molecule of a pure protein is a question that cannot be answered until we can be certain that absolutely pure proteins are secured to start with, and until our methods of effecting its cleavage have been perfected. Vaughan and his co-workers have long maintained that a sensitizing non-poisonous and a non-sensitizing toxic portion are groups of the same molecule, which they are able to obtain

¹ Jour. Infect. Dis., 1913, xii, 341.

² Ztschr. f. Immunitätsf., 1913, 60, 580.

³ Ztschr. physiol. Chem., 1912, 81, 314.

in vitro from animal, bacterial, and vegetable proteins by a method of splitting with sodium hydroxid in absolute alcohol, as described in the chapter on Infection. The toxic intramolecular group is regarded as non-specific, and the same for all proteins, which explains the identity of the symptoms of anaphylactic shock whatever the protein by which it is induced. The non-toxic sensitizing group, however, is specific, although it may not itself be a protein, or at least a biuret body. Whether or not all proteins contain a sensitizing group has not been determined. In keeping with his theory of the rôle of the toxic moiety of a split protein molecule in the production of disease, Vaughan believes that when proteins are introduced parenterally into animals, the non-toxic portion stimulates the body cells to elaborate specific ferments, constituting the phase of sensitization, so that when this protein is subsequently introduced, digestion rapidly takes place with the liberation of the toxic substance responsible for the characteristic symptoms, which may terminate in death.

The correctness of this theory as relating to anaphylaxis has been questioned by Coca,¹ who believes that the process employed may not result in the splitting of all molecules and that mere traces of the original protein in the non-toxic residue sufficient for sensitization, but insufficient for intoxication or desensitization, may explain the experimental results. Zunz² has apparently fallen into the same kind of error in experiments with proteoses of ox-serum from which he concluded that the amino-acid group responsible for sensitization is not the same as that producing shock. In the experiments of Gay and Robertson³ upon this question, globin was found unable to engender the production of anaphylactic and complement-fixing antibodies; a compound of globin caseinate, however, engendered the production of complement-fixing antibody for globin, but did not sensitize to globin. Gay and Robertson have interpreted these results as indicating that the property of specificity is exercised independently by chemical groups in the protein molecules, but their experiments do not indicate that this holds good in anaphylaxis.

The evidence supporting the theory that in a protein substance one part acts as anaphylactogen and other as intoxicating agent or producer of the anaphylactic reaction, is incomplete; very probably both functions are exercised by the same protein molecule.

The investigations of Wells and Osborne⁴ in their study of the anaphylactogenic activity of purified vegetable proteins, either chemically related, but derived from different sources (gliadin of wheat and rye and hordein or barley) or chemically different, but derived from the same source (gliadin and glutenin of wheat), has led them to the opinion that specificity is determined by the chemical structure of the reacting proteins rather than by their biologic origin. They say, however, "Until we have some means whereby the chemical individuality of a protein can be established the possibility will remain that our so-called pure preparations of proteins consist of mixtures, or combinations of proteins which have thus far resisted all efforts to separate them."

These investigators likewise believe that the entire protein molecule is not involved in the specific portion of the anaphylactic reaction, but that this is dependent upon certain groups only and that one and the same protein molecule may contain two or more such groups. Here again, however, the question of the purity of the proteins employed in the experiments may be questioned, and the subject requires further investigation.

¹ Tice's Practice of Medicine, 1920, 113, 114.

² Ztschr. f. Immunitätsf., 1912-13, 16, 580.

³ Jour. Exper. Med., 1913, 17, 535.

⁴ Jour. Infect. Dis., 1913, 12, 341.

Anaphylactogenic Activity of Protein Derivatives, Racemized Proteins, etc.—As previously mentioned, whole proteins possess the greatest degree of antigenic activity; this function is greatly reduced by even the earliest digestion products. Until recently the proteoses (albuminoses) and peptones have been regarded as lacking in antigenic activity; Fink,¹ who gives a good review of the literature, has found some slight evidences of antigenic activity of proteoses, and it is probable that tuberculin anaphylaxis is due to proteose sensitization. As mentioned above, Abderhalden² claims to have sensitized with a synthesized polypeptid containing fourteen molecules of leucin and glycine, and Zunz³ claims success with simpler polypeptids. A large amount of the work conducted with proteoses and peptones (polypeptids) is based, however, upon reactions in the lower animals which may have been produced by the toxicity of the substances themselves, that is, anaphylactoid reactions, to which reference will be made later in more detail. On the other hand, *while the evidence of anaphylactogenic activity of proteoses and peptones is very slight as based upon experiments with the lower animals, it is probable that they may be more active in man, who is more susceptible to anaphylaxis, but with different manifestations than those exhibited by the lower animals.*

The anaphylactogenic activity of proteins is likewise reduced or entirely suppressed for guinea-pigs and dogs by other chemical methods and physical agents. For example, Wells⁴ has found acid albuminate antigenic, but alkali albuminate non-antigenic; Busson⁵ found osmic acid to reduce the antigenic activity of proteins. Ultraviolet rays have been found by Doerr and Moldovan⁶ to reduce antigenic activity. Broeck⁷ found that the racemized proteins of Dakin were non-anaphylactogenic. As a general rule the modified proteins have shown a progressive weakening in antigenic activity, but have preserved biologic specificity; in the investigations of Schittenhelm and Stroebel,⁸ however, with iodized serum and iodized egg-white, it was found that while biologic specificity was preserved, the animals also exhibited an acquired iodine specificity. We shall discuss this phase in more detail in a consideration of the drug sensitinogens.

Non-protein Anaphylactogens.—As with other immunologic reactions, observations have been made that are interpreted as indicating that non-protein substances are capable of producing anaphylaxis; thus Pick and Yamanouchi⁹ sought to demonstrate the antigenic properties of alcohol-soluble constituents of horse- and beef-serum, but conservatively concluded that their results may have been due to a combined action of protein and fat combinations. Similar conclusions were also drawn by Uhlenhuth and Haendel¹⁰ in their study of animal and vegetable oils and fats. Bogomolex¹¹ is less conservative, and believes that he has succeeded in producing lipid anaphylaxis; these claims, however, could not be confirmed by Thiele and Embleton.¹² Meyer¹³ was able to sensitize pigs with pure lipoids extracted

¹ Jour. Infect. Dis., 1919, 25, 97.

² Ztschr. f. physiol. Chem., 1912, lxxxi, 315.

³ Archiv. internat. de physiol., 1919, 15, 179, 192.

⁴ Jour. Infect. Dis., 1909, 6, 506.

⁵ Ztschr. f. Immunitätsf., 1911-12, 12, 671.

⁶ Biochem. Ztschr., 1912, xli, 27.

⁷ Jour. Biol. Chem., 1914, 17, 369.

⁸ Ztschr. f. exper. Pathol. u. Therap., 1912, 21, 102, 108.

⁹ Ztschr. f. Immunitätsf., 1909, 5, 676.

¹⁰ Ztschr. f. Immunitätsf., 1910, iv, 761.

¹¹ Ztschr. f. Immunitätsf., 1910, v, 121; *ibid.*, 1910, vi, 332.

¹² Ztschr. f. Immunitätsf., 1913, xvi, 160.

¹³ Folia Serologica, 1911, vii, 771; Ztschr. f. Immunitätsf., 1914, xxi, 654.

from tapeworms, but was unable to intoxicate them with the same extracts, results that may be understood, since White and Avery¹ have shown that as little as 0.0001 milligram of edestin will serve to sensitize a pig, whereas larger amounts of protein—more than is contained in Meyer's preparations—are necessary to produce intoxication. Finally, the studies of Wilson² and White³ leave no doubt as to the fact that pure lipoids cannot produce anaphylaxis.

It is probable, therefore, that while all anaphylactogens are soluble proteins, not all soluble proteins are anaphylactogens. Some proteins are known definitely to be sensitizers; others are believed with equal sureness not to be, while others still are doubtful owing to the difficulties presented in their preparation in pure form. *It is to be borne in mind, however, that our data is mainly based upon the results of experiments conducted with the lower animals, and in our opinion it has been so clearly proved that the success or failure of active sensitization and the phenomena of anaphylaxis vary according to the species of animal, that negative results observed in experiments with guinea-pigs or other of the lower animals are not necessarily applicable to man.* It may be said that man is more susceptible to allergy in a broad sense than any other animal. All substances known to produce anaphylaxis in the lower animals appear to be anaphylactogenic for man; but the reverse is probably not true, that is, substances non-antigenic for the lower animals may still be sensitizers for man.

I have attempted to summarize our information upon the anaphylactogenic activity of various proteins for the lower animals in the following table; some of those grouped as non-antigenic for these animals may be antigenic for man, and with the simple proteins, primary and secondary protein derivatives, we believe it is justifiable to regard them anaphylactogenic for man until proved otherwise.

ANAPHYLACTOGENIC ACTIVITY OF PROTEINS FOR THE LOWER ANIMALS

Antigenic	{	A. Simple proteins	{	Albumins. Globulins. Glutelins. Prolamines. Albuminoids.	
		B. Conjugated proteins	{	Mucins and mucoids. Hemocyanin. Casein; vitellin. Lipoproteins.	
		C. Primary and secondary proteins	{	Acid albumin. Proteoses (albuminoses) Peptones (polypeptids)	Doubtful.
Apparently non-antigenic	{	A. Simple proteins	{	Histones. Protamines. Gelatin.	
		B. Conjugated proteins	{	Alpha-nucleoproteins. Hemoglobin. Globin.	
		C. Primary and secondary proteins	{	Alkali albumin. Crystallizable amino-acids.	

¹ Jour. Infect. Dis., 1913, xiii, 103.

² Jour. Path. and Bact., 1913, xviii, 163.

³ Jour. Med. Res., 1914, xxx, 383.

Relation of Precipitinogens to Anaphylactogens.—According to Doerr and Moldovan¹ the same substances in a protein antigen produce precipitins and anaphylaxis antibody. Weil² came to the same conclusion in experiments showing that precipitin and anaphylaxis antibody are identical, proposing the name "sensitizin" for the latter. Numerous experiments by others have shown a close parallelism between precipitin and sensitizin production in rabbits and guinea-pigs. While the same antigen appears to produce both antibodies, it cannot be said to be proved that the antibodies are identical, although most evidence points to this conclusion. For example, Burckhardt³ showed that a serum may lose its power for conferring passive anaphylaxis at a time when the precipitin content remains high and unchanged; Weil was able to differentiate between the two properties of immune serum by heating and other procedures, to which we will refer later in the discussion on the nature of the anaphylaxis antibody.

NON-PROTEIN SENSITINOGENS

Allergy to non-protein substances presents one of the most perplexing problems in immunology. Apparently the phenomenon is not confined to the human race. The superpurgation of horses sometimes following the administration of an ordinary laxative dose of aloes resembles an allergic reaction; likewise the intolerance of cattle to mercury. Auer⁴ failed to induce hypersensitiveness in guinea-pigs to salvarsan. When iodine was mixed with guinea-pig-serum Friedberger and Ito⁵ claim to have successfully sensitized guinea-pigs, and Swift⁶ obtained similar results with salvarsan. These results support the theory of Wolff-Eisner that drugs may combine with the blood or tissue proteins and form a protein sufficiently foreign to act as a sensitinogen. On the other hand, hypersensitive persons may react so quickly after swallowing the drug to which they are susceptible or the drug may produce a local skin reaction so promptly when applied to an abrasion, that it would appear that this explanation is inadequate. Furthermore, as pointed out by Doerr,⁷ not all compounds are capable of entering into chemical combination with proteins, and even when they do, the specificity of the original protein appears to be maintained.

Sensitinsogens of this kind from plants (poison ivy, poison oak, poison sumac) have been generally regarded by Acree and Syme,⁸ Adelung,⁹ and others as glucosids; it is possible, however, that traces of protein may be present in these as well as in some preparations of quinin, belladonna, and the like, although all attempts to sensitize the lower animals with them or engender the production of antibodies have failed.

Practically all of the known non-protein sensitinsogens embracing the medicaments and non-medicaments, possess toxicity and more or less constant physiologic activity peculiar for each substance. But the allergic effects are different from the toxic effects and quite similar for all.

The mechanism of sensitization with non-protein sensitinsogens is, therefore, still unknown. The process of sensitization and the allergic reaction is apparently cellular, as will be discussed later with the theories of the

¹ Ztschr. f. Immunitätsf., 1910, 5, 5, 125 and 161.

² Jour. Immunology, 1916, 1, 1.

³ Ztschr. f. Immunitätsf., 1910, 8, 87.

⁴ Jour. Exper. Med., 1911, 14, 497.

⁵ Ztschr. f. Immunitätsf., 1912, 12, 241.

⁶ Jour. Amer. Med. Assoc., 1912, lix, 1236.

⁷ Hand. d. path. org. Kolle-Wassermann, 2d ed., 112, 947.

⁸ Jour. Biol. Chem., 1907, 2, 547.

⁹ Arch. Int. Med., 1913, 11, 148.

mechanism of allergy. *It is possible that non-protein sensitinogens may produce their effects by alteration of cellular proteins of some organs, and notably the skin, with the production of a modified protein sufficiently foreign to engender the production of cellular antibodies.* At any rate the phenomenon apparently results as a reaction in the cells rather than in the body fluids; this altered, exaggerated activity toward the exciting substance aptly expressed as "allergy" is the characteristic change, although nothing is as yet known of the exact nature of this cellular change and how it is produced.

The list of non-protein sensitinogens is quite large and includes the following:

A. Glucosids of various plants¹ as of the Anacardiaceæ embracing *Rhus diversiloba* (poison oak), *Rhus toxicodendron* (poison ivy), *Rhus venenata* (poison sumac), etc.

B. Alkaloids from opium, quinin bark, belladonna leaves, stramonium, hyoscyamus, etc.

C. Essential oils and balsams (cubebs, copaiba, sandalwood), resins, turpentine, etc.

D. Metals, as mercury, arsenic (including salvarsan and neosalvarsan), iron, etc.

E. The halogens, as bromids and iodids.

F. Coal-tar, benzol, and methane derivatives, as antipyrin, iodoform, salicylic acid, creosote, salol, etc.

ACTIVE SENSITIZATION

Active sensitization or allergization is the process of introduction of the exciting agents or sensitinogens into the body cells.

The *route* of administration is usually parenteral, as by subcutaneous, intramuscular, intraperitoneal, intravenous, intracardiac, subdural, or intracerebral injection. Any of these routes of administration may be followed for the experimental production of active sensitization of the lower animals; probably the intraperitoneal route is mostly employed and the time required for bringing about sensitization varies according to the route of administration, as will be shortly discussed. Anaphylaxis of man is commonly acquired by the administration of horse antisera for prophylactic and therapeutic purposes; sensitization may result from subcutaneous, intramuscular, intravenous, or subdural injections.

Sensitization of guinea-pigs has also been effected by instillations of horse-serum into the conjunctival sac, as reported by Rosenau and Anderson,² and by repeated instillations into the nose, as reported by Sewall and Powell.³ These results are of considerable importance in relation to the sensitization of human beings with pollens and other proteins through the mucous membranes of the respiratory tract.

Sensitization may also result from the enteral introduction of sensitinogens, traces apparently being absorbed unchanged through the intact mucous membrane of the intestines or, more often, through injured mucosa. Wells and Osborne have reported successful sensitizations of guinea-pigs by feeding cow's milk, sera, and egg-albumen, and similar experiments have been reported by others. This route of sensitization is of primary importance in relation to allergies of foods and drugs encountered among human beings. Apparently sensitization by this route is especially apt to occur in the young when injuries to the gastro-intestinal tract are most likely to occur.

¹ For a description of plants producing dermatitis venenata consult White's *Dermatitis Venenata*, Cupples & Hurd, Boston, 1887.

² Jour. Med. Research, 1908, 19, 37.

³ Arch. Int. Med., 1915, 16, 605.

The *amounts* of protein sensitinogens required to effect sensitization may be surprisingly small, but varies in some degree with the route of administration and the species of animal. Guinea-pigs are apparently the most readily sensitized; rabbits, cats, dogs, and human beings require large amounts and usually several injections. Sensitization occurs more readily upon intracerebral and intravenous injections than upon subcutaneous, and the "physiologic" avenues of introduction, as the respiratory and gastro-intestinal tracts, are still more uncertain.

Active Sensitization of Guinea-pigs.—The amount of horse-serum required to sensitize guinea-pigs may be as small as 0.000,001 c.c. as reported by Rosenau and Anderson.¹ Uniform sensitization, however, cannot be effected by such amounts. Besredka places the minimum amount necessary to secure uniform results at 0.001 c.c., whereas 0.0001 c.c. proved insufficient in a considerable percentage of animals. The sensitizing dose of horse-serum ordinarily employed in experiments upon guinea-pigs is 0.01 c.c. by subcutaneous or intraperitoneal injection; amounts ranging from 0.001 to 0.1 c.c. are ordinarily followed by an incubation period of ten to twenty days. Larger doses also sensitize, but may require a longer period of incubation. Wells² has successfully sensitized guinea-pigs with as little as 0.000,001 gm. of crystallized egg-albumen and 0.000,000,1 gm. of edestin. The following table gives the average amounts of various protein sensitinogens for guinea-pigs of about 300 gm. weight:

SENSITINOGEN.	SENSITIZATION.		INCUBATION (DAYS).	INTOXICATION.	
	Route.	Dose.		Route.	Dose.
Serum.	Subcutaneous.	0.1 c.c.	16	Intravenous.	0.1 c.c.
Serum.	Intraperitoneal.	0.01 c.c.	10-12	Intravenous.	0.1 c.c.
Serum.	Intracerebral.	0.001 c.c.	8-10	Intracerebral.	0.1 c.c.
Milk.	Intraperitoneal.	1.0 c.c.	20	Intravenous.	0.1 c.c.
Egg-white.	Intraperitoneal.	0.01 c.c.	21	Intravenous.	0.05 c.c.
Crystallized egg-white.	Intraperitoneal.	0.005 gm.	21	Intraperitoneal.	0.05 gm.
Purified vegetable protein.	Intraperitoneal.	0.002 gm.	21	Intravenous.	0.005 gm.
Purified vegetable protein.	Intraperitoneal.	0.002 gm.	21	Intraperitoneal.	0.1 gm.

Active Sensitization of Rabbits, Cats, and Rats.—Rabbits are not as readily sensitized; as a general rule the amounts required are from 1000 to 10,000 times as much of the same substances as suffice for guinea-pigs. Sensitization is usually affected by intravenous or intraperitoneal injections of 1 c.c. of serum per 1000 grams of weight; the former route is preferable. The period of incubation is at least ten days and may be four to six weeks. Much smaller amounts of serum may, however, suffice as occasionally shown by sensitization with traces of serum adhering to corpuscles injected in the preparation of immune hemolysins. Coca has given the following method for the sensitization of rabbits with horse-serum: "Two injections of 1 or 2 c.c. are given by the subcutaneous or intraperitoneal route at an interval of five days. Three days after the second injection daily intraperitoneal or intravenous injections of 0.2 c.c. are made over a period of two weeks or more,

¹ Hygienic Lab. Bull., 1906, No. 29.

² Jour. Infect. Dis., 1908, 5, 449.

and five days after the end of this period the test injection of 2 c.c. is made into the marginal vein of the ear." The same method may be employed for cats, the amounts being the same; likewise for rats, the amounts being about one-tenth less.

Active Sensitization of Dogs.—Dogs are likewise more difficult to sensitize than guinea-pigs. The injections may be given subcutaneously or intraperitoneally in dose of 1 c.c. per 100 gm. of weight; the incubation is usually three to six weeks and anaphylactic shock is best produced by intravenous injection. For producing anaphylaxis in the dog Weil has advised two sensitizing injections of 5 c.c., the first being given subcutaneously, the second intravenously after an interval of several days; the test injection of 20 to 30 c.c. is given intravenously several weeks later.

Incubation Period of Active Sensitization.—This refers to the time required for sensitization to be effected after the sensitinogen has been introduced into the body. It varies with the species of animal, the amount of antigenic material employed and route of its administration, and method of introducing the intoxicating dose of antigen. The shortest period of incubation observed in the guinea-pig was five days; the average is eight to twenty days. In dogs and rabbits from ten to twenty-eight days are generally required.

In human beings the incubation after an injection of serum may be from a few hours to sixteen days. In about 20 per cent. of individuals a reaction (serum sickness) may appear within three days, but the usual period is eight to twelve days. This subject will be discussed in more detail under Serum Sickness.

In drug hypersensitiveness the period of incubation required for sensitization is generally unknown. Hypersensitiveness to arsphenamin and neo-arsphenamin may develop in a few weeks during the administration of repeated doses.

The simultaneous injection of more than one antigen in equal amounts does not influence the incubation periods; if, however, one antigen is given in relatively large amounts twenty-four hours previous to the usual sensitizing injection of a second antigen, the incubation period for the latter is markedly increased (Coca).

Duration of Active Sensitization.—Guinea-pigs are likely to remain sensitized throughout the remainder of their lives, which may be a year or more; according to Coca¹ and Scott² rabbits may lose their hypersensitiveness in two to three weeks. Dogs are likewise apt to lose the hypersensitive state, while human beings are likely to remain hypersensitive to serum, foods, pollens, and drugs for years and sometimes throughout life.

PASSIVE SENSITIZATION

Passive sensitization consists in transferring the allergic condition to a perfectly normal animal by injecting it with the serum from an actively sensitized one.

Historic.—The phenomenon was discovered almost simultaneously and independently by several investigators. Nicolle³ passively sensitized a normal rabbit with an injection of serum from a sensitized animal and produced the Arthus local reaction in the former; Richet⁴ injected a normal dog with the blood of a dog sensitized to mytilocongestin and found that the former became sensitive one or two days later. Otto⁵ and Friedmann⁶

¹ Virchow's Archiv., 1909, cxcvi, 92.

² Jour. Path. and Bacteriol., 1911, 15, 31.

³ Ann. de l'Inst. Pasteur, 1907, 21, 128.

⁴ Ann. de l'Inst. Pasteur, 1907, 21, 497.

⁵ Münch. med. Wchn., 1907, 1665.

⁶ Münch. med. Wchn., 1907, 2414.

succeeded in sensitizing normal guinea-pigs with injections of serum from sensitized animals. Gay and Southard¹ also recorded possible instances of passive sensitization in their early experiments by injecting normal guinea-pigs with the serum of sensitized ones, and finding the former sensitive about ten days later. It is possible, however, that under these conditions the serum carried both antigen and anaphylaxis antibody and that the animals were actively rather than passively sensitized.

Maternal Transmission.—Lewis² showed that the newborn of a sensitized mother guinea-pig may be sensitive, which is an example of homologous passive sensitization. This transmission was found to vary greatly in different young pigs and likewise the duration of the inherited sensitiveness. Otto found that young pigs may remain sensitive for as long as forty-five days after birth. This function of transmitting the condition of sensitization is solely maternal; the male takes no part whatever in the transmission.

Homologous and Heterologous Passive Sensitization.—Sensitization can be passively transferred to individuals of the same species (homologous) or to those of different species (heterologous). Doerr has published the following list of positive and negative results in attempted heterologous passive sensitization:

(a) Guinea-pigs have been passively sensitized with injections of serum from man, monkey, rabbit, dog, cat, and horse sensitized to various proteins. The rabbit with injections of serum from sensitized guinea-pigs and the pigeon with the blood of the chicken.

(b) Negative results have been observed in transferring the serum of birds to mammalia, and the reverse, mammalia to birds; likewise attempts to transfer the sensitiveness of rabbits and guinea-pigs to white mice have failed.

The best example of passive sensitization is the injection of guinea-pigs with the serum of sensitized rabbits, and this combination is recommended for experimental work in this subject. Rabbits excel other laboratory animals in the production of antibodies and guinea-pigs are most satisfactory for passive transfer. Guinea-pigs of equal weight that have received identical amounts of a sensitizing serum passively acquire an almost identical degree of hypersensitiveness to the antigen.

Passive Sensitization with Human Serum.—According to Coca allergies to serum, drugs, pollens, etc., among human beings cannot be passively transferred to guinea-pigs. Coca has termed as true anaphylaxis only such reactions as are due to protein antigens and an antibody capable of conferring passive sensitization. However, this criterion appears to be too rigid; even the serum of guinea-pigs highly sensitized to a foreign protein may contain too few antibodies to confer passive sensitization to other guinea-pigs, and it is possible that the cells of a human being may be highly sensitized without there being sufficient free antibodies in the serum to confer passive sensitization.

Schultz and Larson³ claim to have passively sensitized guinea-pigs with injections of the sera of infants hypersensitive to cow's milk, and Ramirez⁴ has recorded a remarkable instance of accidental passive sensitization of a man by transfusion with the blood of an individual anaphylactic to horse protein.

Bruck⁵ and Klausner⁶ were the first to claim success in the passive trans-

¹ Jour. med. Research, 1907, 16, 143.

⁴ Jour. Amer. Med. Assoc., 1919, lxxiii, 984.

² Jour. Exper. Med., 1908, 10, 1.

⁵ Berl. klin. Wchn., 1910, 517.

³ Archiv. of Pediatrics, 1918, 35, 705.

⁶ Münch. med. Wchn., 1910, 1983.

fer of drug allergies to guinea-pigs, but these results have been questioned, and it is now the consensus of opinion that guinea-pigs cannot be passively sensitized with the sera of human beings hypersensitive to drugs and pollens.

Mechanism of Passive Sensitization.—It is commonly believed that following the injection of serum carrying anaphylactic antibodies, that the latter combine with the body cells and apparently with those concerned in active sensitization. As a general rule a few hours must be allowed for this union with cells to occur, but in the rabbit passive sensitization takes place almost immediately after the intravenous injection of serum. Upon union of the antibodies with cells a similar disturbance in colloidal equilibrium apparently results as occurs in active sensitization so that when the antigen is suddenly brought into relation with the cells, the allergic reaction results.

PREVENTION OF SENSITIZATION AND ALLERGY; ANTISENSITIZATION

Several of the early investigators in allergy sought for means of prevention of sensitization and allergic shock or intoxication by reason of the importance of these subjects in the serum prophylaxis and treatment of disease.

Prevention of Allergic Shock.—Efforts along this line were directed toward the neutralization of the toxic substance in horse-serum, considered at this time responsible for the allergic reaction to this substance. A large variety of substances was employed by Besredka, including alcohol, chloroform, ferments, and various salts, but with negative results; indeed, toxicity of the serum was sometimes increased (serotoxin) and presumably by the auto-digestion of serum proteins, following the removal of antiferment by these substances, as shown by later researches. Furthermore, it is now definitely known that there is no distinct or toxic fraction of serum responsible for the production of allergic shock.

Rosenau and Anderson discovered that boiling horse-serum removed its capacity for eliciting the anaphylactic reaction in sensitized guinea-pigs; Besredka also found that serum diluted with 3 parts of distilled water and heated at 100° C. for twenty minutes was rendered harmless for sensitized guinea-pigs. Even moderate heating at 56° C. for one hour on each of four days in succession was found capable of reducing the shock-producing effects of serum, and this method is practised at the Pasteur Institute in the preparation of therapeutic sera. Higher temperatures are not permissible owing to the destruction of antibodies.

According to Besredka,¹ ether and alcohol narcosis protects sensitized guinea-pigs against allergic shock; Rosenau and Anderson² found that these substances may mask the reaction, but fail to prevent a fatal outcome. Morphin narcosis is without effect. Auer has found the administration of atropin of aid in avoiding severe reactions among sensitized guinea-pigs. Further discussion of this subject is reserved for a succeeding chapter in a consideration of desensitization and prevention of allergy in man.

Prevention of Sensitization; Antisensitization.—As described by Julian Lewis³ if guinea-pigs are injected with large amounts of dog-serum (1 or 2 c.c.) at the same time or within twenty-four hours of the time of injection of the usual sensitizing dose of horse-serum (0.1 c.c.), active sensitization to horse-serum either does not occur at all or to a much less degree than occurs among animals receiving only the horse-serum.

¹ Ztschr. f. Immunitätsf., 1909, 2, 591.

² Ann. de l'Inst. Pasteur, 1908, 22, 465.

³ Jour. Infect. Dis., 1915, 17, 241.

Weil¹ described instances of resistance to passive sensitization in guinea-pigs that had received previous injections of normal rabbit-serum and proposed the term "antisensitization" for the process. Coca has summarized the results of these investigations as follows:

1. The previous injection of rabbit's serum obstructs the passive sensitization with rabbit's immune serum, but not with homologous (guinea-pig's) immune serum.

2. The interference is established after an incubation period, which is longer (eight days) after small injections (0.1 to 0.5 c.c. repeated), than after large injections (1 to 8 c.c.), that is, four days.

3. The duration of the normal period of heterologous passive sensitization in guinea-pigs is six days. If the guinea-pigs have received 0.1 c.c. of normal rabbit's serum two to eight days previously, the period of passive sensitization with heterologous serum is shortened to barely five days. This period may be shortened, also, by the injection of 0.6 c.c. of normal rabbit's serum made on the day following the passively sensitizing injection.

4. The refractory condition of antisensitization persists for at least sixty-eight days.

5. Active sensitization is unaffected by the injection of large amounts of normal rabbit's serum.

Of considerable importance in relation to this subject of antisensitization are the feeding experiments conducted by Wells² with young guinea-pigs. In these investigations it was found that animals raised on oat proteins cannot be sensitized to these proteins, but if raised without oats they may be sensitized. When fed on egg-albumen they are at first sensitized, but if feeding is kept up for a long enough period the animals become refractory and cannot be sensitized. Feeding for as long as ten weeks on bread and milk rendered the animals sensitive to milk, but did not result in desensitization.

Weil explained the phenomenon on the basis of the development and activity of anti-antibodies; another explanation is to the effect that the number of receptors available for anchoring antigen or antibody to body cells as the first step toward active or passive immunization, is limited, and that the large dose of indifferent serum given before or with the injections of sensitizing antigen or antibody serum effectually blocks union of these to cells which otherwise would become actively or passively sensitized. It is highly probable, however, that both of these hypotheses are in error and that the phenomenon is one involving a disturbance of intracellular colloidal equilibrium.

THE PRODUCTION OF ALLERGIC REACTIONS

The amount of sensitinogen required to produce allergic reactions including anaphylactic shock, may be infinitely small for human beings, but in experimental anaphylaxis of guinea-pigs and other of the lower animals, usually more is required than suffices for sensitization. Doerr and Russ³ have stated that in general terms the intoxicating dose is about a thousand times greater than the sensitizing dose. The amount required varies, however, with the route of administration; with intracerebral, intravenous, and intracardiac injections the doses sufficing for the production of shock are much less than required by intraperitoneal and subcutaneous injections. As a general rule the intravenous and intracerebral (Besredka) routes are preferred for the production of acute shock. Subcutaneous injections induce shock more slowly, but are said to yield more specific reactions. *If the*

¹ Ztschr. f. Immunitätsf., 1913, 20, 199; 1914, 23, 1.

² Jour. Infect. Dis., 1911, 9, 147.

³ Ztschr. f. Immunitätsf., 1909, 2, 109.

sensitino-gen is known to be toxic and capable of producing anaphylactoid phenomena upon intravenous injection due to intravascular agglutination and hemolysis, with thrombosis and embolism, the intraperitoneal route is to be preferred.

Allergic reactions may be elicited by applying the sensitinogen to mucous membranes as in the conjunctival tuberculin reaction; likewise the inhalation of pollens and the effluvia of the lower animals suffices for inducing attacks of hay-fever and allergic asthma in sensitized individuals. The application of these substances to abrasions of the skin may elicit local reactions and indicate that the amounts of sensitinogens required to elicit local and general allergic reactions may be infinitely small.

Allergic reactions may be elicited by absorption of the exciting substance from the intestinal tract, as shown by the food allergies. Indeed, in buck-wheat allergy a reaction may be elicited by dropping small amounts on the tongue. Apparently absorption does not occur from the stomach and reactions are likewise infrequent following rectal injections; reactions are likely to follow when the substance has reached the small intestine.

THEORIES OF THE MECHANISM OF ALLERGY

Having reviewed our knowledge bearing upon the nature of the sensitinogens or exciting substances of allergy, the symptoms, lesions, and pathologic physiology of the allergic reaction, we now arrive at the most perplexing portion of the subject, namely, a discussion of the nature of the mechanism of allergy. The subject has commanded the attention and efforts of numerous investigators and a great mass of data has been accumulated, but the exact nature of allergy cannot be said to have been elucidated and, as is usual under these conditions, numerous theories have been proposed. I shall briefly present some of these theories and then proceed to discuss the more important data bearing upon this subject, which, I believe, shows that *allergy is a cellular colloidal reaction* and to be explained on a basis of physical chemistry.

The Humoral Theories of Allergy.—These include the following:

1. *Richet*¹ held that the sensitizer, or anaphylactogen, contains a substance which he called "congestin" (because he did his original work with extracts of the tentacles of sea anemones, which are toxic and produce congestion of the internal organs), and that this generates in the animal another substance, known as the "toxogenin." The reaction between the latter and the homologous protein on reinjection sets free a poison, "apotoxin," which, because of its effect on the nervous system, produces the symptoms of anaphylaxis. This theory is practically the same as that generally accepted today, except that the antigen is not of necessity primarily toxic for the animal.

2. *Hamburger and Moro*² suggest that the first injection leads to the formation of precipitins, and that on reinjection precipitates are formed; these, they contend, may, by the formation of capillary emboli, produce acute anaphylaxis, or at least that precipitin formation runs parallel with the antibody formation. The symptoms of anaphylaxis, however, are not those of embolism, and there is no evidence to show that precipitation occurs *in vivo*, although, as Zinsser points out, precipitins may play the rôle of sensitizers of the antigen, preparing them for final lysis or cleavage by a complement. In other words, the precipitin would act as an amboceptor, differing, however, from our general conception of the nature of amboceptors

¹ Ann. de l'Inst. Pasteur, 1908, 12, 465.

² Wien. klin. Wchn., 1903, 445.

by being active in the absence of complement, unless precipitation is a secondary physical phenomenon in the nature of a colloidal reaction.

3. *Besredka*¹ taught that the sensitizer contains two substances—"sensibilisinogen" and "antisensibilisin." When injected the first time the former develops in the body a substance called "sensibilisin," and on re-injection the sensibilisin and antisensibilisin continue to form a poison that acts on the nervous system.

4. *Gay and Southard*² have stated that, as a result of the first injection of serum, there remains in the circulation a protein substance called "anaphylactin," which is slowly absorbed and continues to stimulate the cells, leading to an abnormal affinity for the homologous protein, which, on re-injection, leads to anaphylactic shock.

5. *Vaughan and Wheeler*³ are of the opinion that, with the parenteral introduction of a foreign protein, the body cells are stimulated to produce a specific zymogen or ferment that digests it. The protein of the first injection is so slowly digested that the effects are not recognizable. After the protein of the first injection has been disposed of, the new ferment continues to be formed in the cells, and on the second injection, after the proper interval has been allowed to elapse, this zymogen is activated and splits up the protein, which promptly and abundantly results in the production of the symptoms of anaphylactic shock. Vaughan believes that there is a non-specific poisonous group or moiety in each protein molecule which, when liberated by the ferment, is responsible for anaphylaxis. This poisonous group is held as being the same in all proteins, and hence the similarity of lesions and symptoms of anaphylactic intoxication in animals regardless of whether the protein is of animal, vegetable, or bacterial origin. The nature of the ferment is not clear. In 1907 they regarded it as a zymogen—a theoretic labile chemical body resulting from intramolecular rearrangement in the protein molecules of the cell. Little is known of the action of these ferments except that in some manner they cause cleavage of the protein molecule and liberation of the toxic moiety. Later, Vaughan speaks of the ferment as consisting of an amboceptor and a complement, the ferment (presumably the complement portion) being inactivated by a temperature of 56° C. and reactivated on the addition of serum and organic extracts. Although Vaughan's theory best explains the nature and source of the anaphylactic poison, that of Friedberger explains the production of the "ferment," or rather the protein sensitizer (amboceptor), which, with a complement, digests the protein and sets free or produces the protein poison.

6. *Friedberger*⁴ has attempted to explain anaphylaxis on the basis of Ehrlich's side-chain theory of the action of antigens and the production of antibodies similar to toxin-antitoxin immunity. This theory assumes that, on the first injection, the protein finds but few groups of cellular receptors with which it can combine, and for this reason it is not poisonous. During the period of incubation the animal cells develop receptors specific for the homologous protein; with a single small dose of protein most of these receptors remain attached to the cell (sessile); on repeated injections, the newly formed receptors are in large part cast off into the blood, and constitute the precipitins. In this manner an animal relatively insusceptible to a foreign protein is rendered highly susceptible, and on the second injection the protein is anchored firmly to the cell, just as the cells of an animal may

¹ Compt. rend. Soc. de biol., 1907, lxxiii, 294; 1909, 21, 384.

² Jour. Med. Research, 1907, 16, 143; 1908, 18, 407; 1908, 19, 1, 5, 17.

³ Jour. Infect. Dis., 1907, 4, 476.

⁴ Ztschr. f. Immunitätsf., 1909, 2, 208; 1909, 3, 692; 1910, 4, 636.

anchor diphtheria toxin. One of the essential features of this theory is that it assumes that, ordinarily, the receptors are not preformed in sufficient numbers to anchor enough protein to injure the animal with the first injection, regardless of the size of the dose. On the other hand, tetanus and diphtheria toxins find large numbers of sessile or cellular receptors, and are highly toxic on the first injection. As originally evolved, the theory did not explain the nature of the toxic agent responsible for the lesions and symptoms of anaphylaxis, and made no mention of the protein poison. Nevertheless it affords the best explanation we have on the formation of the "ferment" or protein sensitizer (amboceptor). At one time Friedberger believed that anaphylaxis could be explained on the basis of a precipitin reaction. Anti-anaphylaxis was explained on the assumption that the protein of the reinjection uses up the sessile receptors already developed, and, accordingly, not enough are present at the end of the period of incubation to produce anaphylactic shock. Passive anaphylaxis was explained on the ground that the free receptors in the blood of a sensitized animal become, on injection into a fresh animal, anchored to the cells, thus forming fixed or sessile receptors that anchor the protein on reinjection and lead to anaphylaxis.

*Nolf*¹ has proposed a theory of anaphylaxis that has come to be known as the "physical theory." It assumes that the active constituent of proteins is a thromboplastic substance that disturbs the colloidal equilibrium of the blood and leads to the deposition, on the surface of the leukocytes and the endothelial cells of capillaries, of a delicate film of fibrin. Thus stimulated, the cells pour out an unusual amount of antithrombin. On account of the consumption of a part of the fibrinogen and the increased formation of antithrombin the blood fails to coagulate after anaphylactic shock or peptone poisoning. Owing to the coagulation deposits on the endothelial cells, the viscosity is increased and the leukocytes adhere to the vessel walls, thus accounting for the leukopenia observed after protein injection. The endothelial cells are injured, and the walls of the capillaries become more readily permeable, thus accounting for the local edema often seen in anaphylaxis. The fine capillaries of a given area may be occluded by thrombi, thus explaining the necrosis characteristic of the Arthus phenomenon. The irritation of the endothelial cells extends to the smooth muscle, leading to vasoparalysis and the characteristic fall in blood-pressure. The affinity of the endothelial cells for the protein is stimulated by the first injection, and acts in a fulminating way on reinjection, thus explaining the suddenness of anaphylactic shock.

The theory, therefore, also assumes the formation of a ferment that acts primarily upon the proteins of the blood, leading to the formation of fibrin, which, as it were, mechanically induces the lesions and symptoms of anaphylaxis. While it offers a plausible explanation, the theory is not well supported, and at best may be regarded as a modification of Vaughan's theory, demonstrating one way in which the protein poison may act.

The Cellular Theory of Allergy.—Many investigators have attempted to explain anaphylaxis on the basis that the reaction is a cellular one, that is, that the antibody is within the cell and that the antigen-antibody reaction occurs in this position rather than in the blood-stream by means of free or circulating antibody and antigen. According to the "cellular theory," if the serum of an immunized animal containing the anaphylactic antibody is injected into a normal animal (passive anaphylaxis) and is followed by an injection of the antigen, an anaphylactic reaction cannot occur before the

¹ Arch. internat. de physiol., 1910, 10, 37; Bull. de l'Acad. roy. de Belg., 1910.

elapse of sufficient time for the antibody to become anchored to cells. The "humoral theory," on the other hand, assumes that the antigen meets the antibody in the blood-stream and explains the time required between the injection of immune serum and antigen in passive anaphylaxis as due to a failure of rapid union between antigen and antibody unless qualitative relations between the two are accidentally correct.

The early theory of Friedberger,¹ explaining anaphylaxis on the basis of "sessile receptors"; the experiments of Friedberger and Girgolaft,² who passively sensitized normal animals by transplanting the thoroughly washed organs of a sensitized animal; the transfusion experiments of Pearce and Eisenbrey,³ who transferred the blood of a sensitized animal to a normal animal and the blood of a normal animal to a sensitized one, finding that the latter, but not the former, reacted when the antigen was injected as soon as the transfusions were completed; the work of Coca,⁴ who found that sensitized guinea-pigs would still react after being thoroughly bled and perfused with salt solution; the investigations of Schultz,⁵ Dale,⁶ and particularly of Weil,⁷ showing that the excised and washed muscles of sensitized animals would react *in vitro* in a bath of Ringer's solution when the antigen was added (Fig. 151), support most strongly the cellular theory of anaphylaxis as emphasized also in the work and recent communications of Doerr.⁸

In the opinion of Weil, Doerr, Bayliss, Coca, and others the "cellular" theory is the only tenable one today. According to this theory of anaphylaxis, the antibody is in or on the body cells; upon union with the antigen the cells undergo a physical shock which has been likened to an electric shock, and this constitutes the basis of the anaphylactic reaction without the formation of any intermediate or chemical poison.

As emphasized by Weil⁹ there is no direct evidence of the production of a chemical poison or anaphylatoxin during or after an anaphylactic reaction in the living animal. This poison has not been satisfactorily demonstrated in the blood and in animals recovering from a general anaphylactic reaction, the phenomena being more suggestive of a transitory "shock" than of an intoxication with a chemical poison. (See pages 632 and 633).

ANALYSIS OF DATA BEARING UPON THE MECHANISM OF ALLERGY

The exciting agents or sensitinogens have been discussed and grouped into two broad classes: (a) the proteins embracing not only the whole molecule, but the primary and secondary derivatives down to and including the proteoses; (b) the non-protein agents. Some of the agents grouped in the latter, as the glucosids and drugs of vegetable and plant origin, may possibly owe their sensitizing properties to traces of protein; others, and especially synthetic compounds, are certainly not proteins, but whether or not they are capable of forming combinations with body proteins and altering these sufficiently to act as sensitinogens is still an open question. At any rate an acceptable explanation of the mechanism of allergy must, in the writer's opinion, cover both kinds of sensitinogens.

¹ Ztschr. f. Immunitätsf., orig., 1909, 11, 208.

² Ibid., 1911, ix, 575.

³ Congr. Amer. Phys. and Surg., 1910, viii, 402.

⁴ Ztschr. f. Immunitätsf., orig., 1914, xx, 622.

⁵ Jour. Pharmacol. and Exper. Therap., 1910, 1, 549.

⁶ Jour. Pharmacol. and Exper. Therap., 1913, iv, 167.

⁷ Jour. Med. Research, 1914, xxx, 87.

⁸ Ergebnisse der Immunitätsf., Reichhardt, Berlin, 1914, 1, 257.

⁹ Proc. Soc. Exper. Biol. and Med., 1915, xiii, 23 (1087).

The Relation of Antibodies to Allergy; Terminology.—Is an antibody concerned in the mechanism of the allergic reaction? The demonstration that sensitization may be passively transferred to animals of the same or different species (passive allergy), answers this question affirmatively. Failure to demonstrate passive sensitization may be due to technical errors or more especially to the absence of antibody from the blood or serum at the time of transfer or the presence of insufficient amounts, but does not necessarily indicate that antibodies are absent as they may be attached to the body cells. This must be true if we accept the contraction of the thoroughly perfused sensitized uterus or lung of the guinea-pig *in vitro* as an allergic reaction. Furthermore, hypersensitiveness may persist for long periods of time, whereas antibodies *in the serum* tend rapidly to disappear; for this reason and because of frequent failure to demonstrate antibodies by the precipitin and complement-fixation reactions, Gay and Southard do not regard allergy as an antigen-antibody reaction. In other words, if there is an allergic antibody then the experiments of the cellularists show that it may be in part, at least, in certain cells or attached to them. For this reason failure of passive sensitization or detection of antibodies *in vitro* cannot be accepted as evidence against a phenomenon being anaphylactic.

Various names have been proposed for this antibody. Richet named it toxogen, believing it responsible for the production of a poison. Otto spoke of it as *reaction body*, Nicolle as an *albuminolysin*, Besredka as *sensibilisin*, von Pirquet as *allergin*, and Weil as *sensibilisin*. It is most commonly designated as *anaphylactin*. In my opinion the terms *allergin* or *sensitizin* are most appropriate because they convey the fundamental meaning of altered and exaggerated activity of cells and sensitization.

This antibody is regarded as a precipitin by some investigators, as a "ferment" by others, and by others still as an albuminolysin or proteolysin, that is, a protein amboceptor requiring the co-operation of a complement.

The Relation of Precipitin to Allergin (Sensitizin).—Several investigators, as Friedberger, Doerr, and Russ, Weil, Wells, and others, have shown that sensitization of guinea-pigs and other of the lower animals with various proteins of animal and vegetable origin is followed by the appearance of precipitins in the blood, together with the capacity for conferring passive sensitization. Although Weil found that heating immune serum at 70° C. for thirty minutes destroys its precipitating power while its capacity for passive sensitization persists, he did not interpret this as evidence of a separation of precipitin and sensitizin, but that the precipitoids so formed (thermostabile haptophore portions only) are essential to passive sensitization. As previously discussed in the chapter on Precipitins, Weil found that the precipitates formed by mixing immune serum and antigen served for active and passive sensitization, that is, the precipitate carries down both whole antigen capable of active sensitization and precipitin, regarded as the passively sensitizing agent.

In serum allergy of human beings usually caused by injections of horse-serum precipitins may be found in the serum, although passive transfer of this kind of allergy has generally failed. Longcope and Rackeman¹ believe that precipitins are always present in those subject to this allergic reaction and that the symptoms and lesions occur until all circulating antigen has been neutralized. C. W. Wells,² however, in agreement with von Pirquet and Schick, was unable to show a constant coincidence of the onset of symptoms of serum allergy with either the appearance of precipitin in the blood, its concentration, or disappearance.

¹ Jour. Exper. Med., 1918, 27, 341.

² Jour. Infect. Dis., 1915, 16, 63.

With protein sensitinogens it would appear, therefore, that precipitin and sensitizin are closely associated even though they cannot be regarded as identical; especially is this true of anaphylaxis in the guinea-pig and rabbit. In serum allergy of human beings precipitin may be found in the serum, while passive sensitization fails, indicating in a general way that sensitizin is absent and the two antibodies separate and distinct. In the other allergies of human beings due to protein and non-protein sensitinogens, precipitins have not been found and passive sensitization has uniformly failed. Finally, Longcope¹ has recently found that in spite of the fact that the white rat could not be made anaphylactic to horse-serum, the tissues of these animals reacted with horse-serum to form precipitins in fair concentration, and the antigens disappeared from the circulation soon after the precipitins reached their greatest concentration in the blood. These experiments indicated, therefore, that in the white rat anaphylaxis and precipitin formation are independent and represent different types of immunologic processes.

The Relation of Albuminolysins or Proteolysins (Amboceptors and Complement) to Allergen (Sensitizin).—Friedmann² observed the development of toxic substances in normal rabbit-serum brought in contact with sensitized ox-blood corpuscles before hemolysis occurred, which produced anaphylactiform symptoms upon intravenous injection into guinea-pigs. Since then Friedberger³ and others have sought to explain the anaphylactic reaction on the basis of the production of a poison designated as “anaphylatoxin” from the antigen by the action of protein amboceptors (albuminolysins or proteolysins) and a ferment (complement). This work is offered as one explanation for the humoral theory of anaphylaxis, the other explanation being based upon a similar production of a protein poison by the activity of serum ferments (proteoses).

If precipitins are identical with protein amboceptors, as maintained by Zinsser and others, the rôle of proteolysins in the production of anaphylactic shock is strengthened, but several objections have been raised against this theory of the mechanism:

1. Weil⁴ and others have failed to demonstrate the poison in the blood of animals during or immediately after anaphylactic shock.

2. Loewit and Bayer⁵ are said to have produced anaphylactic shock in animals previously deprived of complement by the injection of hypertonic saline solution (Hektoen); however, intracellular complement may have been available and the work requires more confirmation before being acceptable.

3. The proof of the ferment activity of complement is almost totally lacking.

4. If this theory is correct, the simultaneous injection of a normal animal with sensitinogen and the serum of a sensitized animal carrying sensitizin should result in immediate symptoms because the anaphylactic reaction may develop within a few seconds. As a matter of fact, such results have been claimed by Biedl and Kraus⁶ in guinea-pigs when they injected intravenously mixtures of horse-serum and the serum of sensitized guinea-pigs; Briot⁷ and Gurd⁸ also obtained similar results, but such experiments have failed or yielded indefinite results in the hands of others.

¹ Jour. Exper. Med., 1922, 36, 627.

² Jour. Immunology, 1917, 2, 399.

³ Arch. f. exper. Path. u. Pharmacol., Suppl. Bd., 1908, 355.

⁴ Jour. Immunology, 1917, 2, 399.

⁵ Arch. f. exper. Path. u. Pharmacol., Suppl. Bd., 1908, 355.

⁶ Ztschr. f. Immunitätsf., 1910, 4, 115.

⁷ Compt. rend. Soc. de biol., 1910, lxxviii, 402.

⁸ Jour. Med. Research, 1914, 31, 205.

5. A similar poison has been produced *in vitro* by Doerr and Russ¹ and numerous other investigators by sera deprived of complement.

6. Many of the experiments offered in confirmation of this theory have been based entirely upon the production in guinea-pigs and rabbits of symptoms resembling anaphylaxis without excluding the possible effects of embolism and thrombosis capable of exciting anaphylactoid reactions.

7. The immediate contraction of perfused sensitized guinea-pig uterus *in vitro* by the Schultz-Dale method, when antigen is added to the bath, cannot be reconciled with this theory that the exciting agent is a product of proteolysis.

8. Similar poisons have been produced *in vitro* by normal serum and inert substances and in the absence of antibodies and antigen. Friedberger claims that under these conditions natural proteolysins (amboceptors) and traces of protein in the substitute antigen may be operative, but these claims cannot have a basis in fact with such a substance as kaolin. This is discussed in more detail in the following paragraphs.

The Relation of Ferments to Allergen (Sensitizin).—The word "ferment" has been loosely employed in connection with immunologic phenomena in general and with anaphylaxis in particular. Vaughan and Wheeler believe that in anaphylaxis the first injection of antigen engenders the production of a "ferment" capable of digesting the antigen upon subsequent injection, with the production of a poison similar in effects to that produced *in vitro* by splitting with alcoholic potash. As mentioned above Friedberger and his school speak of the "ferments" in normal and immune sera and apparently refer to thermolabile complement and protein amboceptors or sensitizers.

Jobling and Petersen speak of a proteolytic ferment in serum as protease and this is the meaning adopted in this discussion. These investigators² have produced protein poisons *in vitro* similar to Friedberger's anaphylatoxin by chemical methods and in such manner as apparently rules out the influence of the thermolabile and easily destroyed complement or alexin. According to their views, serum complement and normal serum proteases are not identical and, whereas the former may be inactivated by heating at 56° C. for half an hour, the latter are more resistant, the increased digestive power observed following the addition of fresh serum being ascribed to the amounts of protease thereby added.

Therefore, while the rôle of complement in the production of poisons in serum may be ruled out, the question remains whether or not anaphylaxis is caused by an increase of proteases and if the latter constitute the sensitizins capable of conferring passive immunization? A great deal of work has been done on these problems, but very little of it is of use in the present discussion. For example, anaphylaxis is highly specific and the poison should be correspondingly specific, that is, its source should be the protein of the sensitinogen, although its properties may be the same irrespective of source since the phenomena of anaphylaxis in any given species are the same for different sensitinogens. But the experiments of Keysser and Wassermann,³ Bordet,⁴ Jobling and Petersen,⁵ Plaut,⁶ Peiper,⁷ Friedmann and Schonfeld,⁸ Bronfenbrenner,⁹ and others, tend to show that the mechan-

¹ Centralbl. f. Bakteri., 1912, lxiii, 243.

² Jour. Exper. Med., 1914, 19, 459, 479.

³ Folia Serolog., 1911, 7, 593.

⁴ Compt. rend. Soc. de biol., 1913, lxxiv, 877.

⁵ Loc. cit.

⁶ Münch. med. Wchn., 1914, lxi, 238.

⁷ Deutsch. med. Wchn., 1914, xl, 1467.

⁸ Berl. klin. Wchn., 1914, li, 348.

⁹ Jour. Lab. and Clin. Med., 1916, 1, 573.

ism is not quite so simple and direct, and that a protein poison may be produced in the absence of the specific antigen. They have demonstrated by experiments *in vitro* that such inert substances as kaolin, barium sulphate, agar or indifferent bacteria or precipitates may replace the antigen in the production of a protein poison. Since the presence of protein substances could be excluded, as with such inorganic substances as kaolin and barium sulphate, the conclusion was naturally drawn that the matrix of the poison was not the antigen or substrate, but the constituents of the serum itself. Jobling and Petersen advanced the theory that the proteolytic ferment was held in check by antiferments (largely the unsaturated fatty acids in serum), and that these substances, as kaolin or barium sulphate, absorb the antiferments and thereby release the proteolytic ferments which proceed to digest the protein of the serum. For this reason these investigators have applied the name "serotoxin" to the protein poison as indicating its source.

A very extensive and valuable investigation by Novy and De Kruij¹ have corroborated and extended these observations and shown, in addition, that informed substances, as peptone solutions and water and even mere coagulation of the blood, may render a serum toxic.

Applying these observations and views to the subject under discussion, it would appear necessary to infer that the antigen is non-specific and acts as it were in a purely mechanical manner; this does not at all agree with the great mass of data showing the highly specific nature of anaphylaxis, and Bronfenbrenner explains the specific production of anaphylatoxin by non-specific ferments as follows:

Specific antibodies are produced, and the combining of these with the antigen causes a falling out or inactivation of the antiferments of the serum by a change in colloidal conditions resulting in the release of normal or non-specific proteolytic ferments which disrupt or digest the protein of the serum. In other words, specific antibodies are produced, but instead of these digesting the protein of the antigen or of the serum directly, they act by uniting with the antigen, and this union results in the removal of anti-enzyme, thereby releasing or rendering active the normal and non-specific enzymes of the serum which produce a protein poison or anaphylatoxin, by digesting the protein of the serum.

However, practically the same objection listed above against accepting the production of a poisonous protein substance by the interaction of complement and specific protein amboceptors, can be offered against the production of anaphylaxis by these poisons produced by the activity of proteases. In other words, the poison has not been satisfactorily demonstrated in the blood of anaphylactized animals, the latent period of passive sensitization is not explained, the possibility of the production of anaphylactoid symptoms by embolism and thrombosis has not usually been sufficiently controlled and the immediate production of anaphylactic contraction of the perfused sensitized guinea-pig uterus by the Schultz-Dale method when the antigen is added to the bath, is far too rapid for proteolysis.

On the other hand, these proteases may be important and active in the production of pseudopositive skin reactions which I will discuss in relation to the local allergic reactions.

The Relation of Humoral Poisons to Allergy.—Setting aside for the present the question of the nature of the antibody concerned in allergy, that is, whether it is a proteolysin of the nature of an amboceptor or a proteolytic ferment, the important question remains whether or not a poison of protein

¹ Jour. Infect. Dis., 1917, 20, 499-854.

origin or otherwise is produced in the blood responsible for the allergic reaction. According to the humoral theories such a poison is produced and is regarded as the direct cause of the allergic reaction; according to the cellular theory such poisons are not produced and have no essential relation to allergy.

The evidence for and against the production of humoral poisons and their relation to allergy may be summarized as follows:

1. The presence of poisons in the blood during and after anaphylactic shock has not been conclusively demonstrated. Novy and DeKruif found that the blood of guinea-pigs sensitized with large amounts of egg-white or horse-serum and shocked by the intravenous injection of the respective antigens, were more toxic than the blood of normal guinea-pigs transfused under similar conditions of transfer time. The amounts of the antigens injected were many times larger than those ordinarily employed in the classical anaphylaxis experiment and the authors state as their opinion that "the antigen is in nowise the source of the anaphylatoxin which is brought into being in shock." As previously stated, Weil obtained uniformly negative results when normal dogs were bled and transfused with a volume of blood equal to or greater than the amount removed from dogs dying or dead in anaphylactic shock.

This subject requires further investigation. It may be, as Dale¹ says, that the poison is produced within the cells and need not be large enough to be detected with the methods at our disposal.

2. The almost total lack of incubation period following the intravenous injection of antigen into sensitized animals and the development of the lesions and symptoms of shock, is evidence against the production of a poison, unless the amount required to produce a reaction is infinitely small. The same may be said of anaphylactic experiments *in vitro* with strips of uteri from sensitized guinea-pigs after the method of Schultz and Dale, when the addition of antigen to the bath is followed almost immediately by vigorous contraction. These experiments appear to rule out the possibility of even an intracellular digestion of a sensitized antigen by antibody or ferment.

3. The production of symptoms of anaphylaxis following the simultaneous intravenous injection of antigen and antiserum into normal animals supports the theory of production of humoral poisons. Results of this kind have been reported by Gurd and by Thiele and Embleton.² In the experiments of Manwaring and Kusama³ employing guinea-pig's lung, it was found that perfusion with a mixture of antigen and the blood of a sensitized animal caused a bronchial spasm. Weil also found that the simultaneous injection of antigen and antiserum was sometimes followed by mild symptoms of anaphylaxis, but the significance to be attached to these results is questioned by reason of similar results being observed by him with intravenous injections of antiserum and an unrelated antigen.

4. Against the theory of production of poisons by humoral antibodies at least, are the experiments of Doerr and Pick, who observed fatal anaphylactic shock in rabbits upon reinjection of the antigen at a time when circulating antibodies had disappeared from the blood. Weil also found that the delayed form of anaphylactic shock in guinea-pigs may be elicited by the intraperitoneal injection of antigen at a time when sensitizing antibodies could not be found in the blood. However, these experiments do not

¹ Jour. Pharm. and Exper. Ther., 1912-13, 4, 167.

² Jour. Immunology, 1916-17, 2, 109.

³ Ztschr. f. Immunitätsf., 1913, 20, 159.

prove that poisons may not be produced by antibodies in or upon the cells; they do indicate, however, that shock may be produced in the apparent absence of circulating antibodies and to this extent reduce the chances of poison production in the blood.

5. The experiments of Weil indicating that the presence of antibodies in the blood of sensitized animals may actually protect against anaphylactic shock by preventing the injected antigen from reaching sensitized cells, is to be interpreted as evidence against the production of humoral poisons by antibodies. Further reference to these observations will be made in the sections dealing with anti-anaphylaxis and desensitization.

6. The isolation experiments of Schultz, Dale, Weil, Coca, and others conducted with strips of uteri, lungs, or other organs from sensitized guinea-pigs, suspended in an oxygenated bath and made to undergo almost immediate and spectacular contraction upon the addition of antigen to the bath, appears to be almost indisputable evidence against the production and activity of humoral poisons, and especially so, since these experiments have been conducted by Dale and others with organs thoroughly perfused with saline solution and washed free of blood.

7. The latent period of passive sensitization is evidence against the production of humoral poisons by proteolysins or ferments. When large amounts of serum from a sensitized rabbit are injected into normal guinea-pigs, a certain period must elapse before the injection of antigen elicits an anaphylactic response. Test injections undertaken during this period have shown that no amount of antibodies in the blood alone can bring about the reaction of anaphylactic shock.

8. That anaphylactic shock may be produced by protein poisons is supported by the fact that the intravenous injection of peptone and sera rendered toxic *in vitro* by admixture with various substances, is frequently followed by anaphylactiform lesions and symptoms. Too much importance, however, cannot be placed upon these results for several reasons, as follows: (a) The symptoms may be anaphylactoid and due to intravascular agglutination, hemolysis, or to embolism and thrombosis. (b) The production of these toxic sera by simple contact with filters, mixture with kaolin, starch, agar, and so forth, has no analogy to anaphylaxis which is a highly specific reaction in which the antigen or exciting agent is specific. (c) The amount of nitrogenous poisons produced *in vitro* required to engender anaphylactiform symptoms is many times greater than the maximum amounts that could possibly be produced in the classical anaphylaxis experiment, as, for example, the fatal outcome produced by the intravenous injection of sensitized guinea-pigs with 0.001 c.c. serum or 0.00005 gm. of crystalline egg-white.

Of the toxic substances of protein cleavage producing anaphylactiform lesions and symptoms, histamin and methyl guanidin are the best examples. Histamin not only produces bronchial spasm in guinea-pigs, obstruction to pulmonary circulation in rabbits, and fall of blood-pressure in dogs, but causes marked urticaria when applied to the skin, resembling the local allergic reaction. Dale has found that it causes the contraction of desensitized strips of uteri and this is interpreted as indicating that it represents a separate substance from that concerned in the anaphylaxis reaction, but this is not necessarily the case, inasmuch as the failure of desensitized tissue to react upon the addition of antigen may be due to exhaustion of antibodies in the cells. Histamin also does not produce the temperature reactions of anaphylaxis or the reduced coagulation time of the blood, but these effects in anaphylaxis may be due to other substances.

The Cellular Colloidal Nature of Allergy.—The sum total of the evidence in favor of *humoral* poisons being the cause of anaphylactic shock is very slight. That toxic products of protein cleavage may be produced *in vitro* which upon injection into animals produces lesions and symptoms closely resembling anaphylaxis is well established. The possibility of such toxic substances being produced in the living animal by disturbances in the equilibrium of plasma colloids is to be granted. It is entirely likely that such poisonous substances may be produced in the blood in anaphylaxis, but the evidence that anaphylactic shock is due primarily to their production and activity is inconclusive. The mere fact that their physiologic effects resemble the lesions and symptoms of anaphylaxis does not establish that they are identical. Such humoral poisons may be produced in anaphylaxis and probably are to some extent under certain conditions, but their production is of secondary importance. Their effects may add to the lesions and symptoms of anaphylaxis as in the production of temperature changes, alterations in the corpuscular elements and coagulation time of the blood, the increase of amino nitrogen, the production of the non-specific phase of local allergic skin reactions, and so forth, but in the writer's opinion there is no proof that these humoral poisons are responsible for the primary and essential lesion of anaphylaxis, namely, the contraction of smooth muscle in man, rabbit, and guinea-pig, changes in the liver cells in dogs, and so forth.

That *cellular* poisons of like nature may be produced commands more attention, but the immediate and vigorous contraction of the washed-out and excised uterus of a sensitized guinea-pig *in vitro* renders this almost impossible of acceptance. The anaphylactiform effects noted upon the excised lung of a guinea-pig perfused with a mixture of antigen and antiserum may be due to non-specific production of toxic substances by disturbance of plasma colloids; this is especially likely in view of experimental data showing how very slight and insignificant manipulations of the blood may render it toxic.

Just what occurs in sensitized cells responsible for the allergic reaction is unknown. We know nothing of what sensitization consists of beyond that it may be accompanied by antibody production. These antibodies may be found in the blood and for protein antigens may be precipitins; on the other hand, they may be purely cellular as shown so conclusively by Block in the grafting of a patch of skin from a person hypersensitive to iodoform to a normal individual. In many forms of allergy the antibody is entirely cellular, as in allergies to tuberculin, other bacteria, foods, and drugs.

Nothing is definitely known of the exact nature of this antibody. With protein antigens as serum and egg-albumen, it is produced in the lower animals in such abundance as to be found free in the blood and capable of engendering passive sensitization. In man sensitized to serum, free antibody is generally not found in the serum, its presence in the blood apparently varying according to the species. Whether it is an intracellular and extracellular precipitin is not definitely known. At any rate the antibody is not to be found in the blood or serum of all allergies; it is essentially and primarily located in the tissue-cells and principally in the cells of involuntary muscle.

The allergic reaction with both protein and non-protein sensitinogens is cellular and apparently caused by alterations in colloidal equilibrium within the protoplasm of the cells carrying the antibody (sensitized cells). As stated by Dale, the reaction can probably be accounted for by the antibody bearing such a relation to the antigen that when they meet a disturbance of the conditions of colloidal equilibrium is set up in the sensitized cells.

As discussed in the chapter on Precipitins the phenomenon of precipitation is apparently a colloidal reaction. Under certain conditions actual intracellular precipitation may initiate allergic shock by changes in the state of aggregation of colloidal particles, but it is not necessary to assume that the quantitative relations of antigen and antibody must be such as to yield visible precipitation.

Briefly, in the writer's opinion, allergy embracing hypersensitiveness to both protein and presumably non-protein substances is a cellular rather than a humoral reaction; the exact nature of the cellular disturbances responsible for sensitization and the production of acute and chronic allergic shock or reactions is unknown, but very probably a phenomenon in the domain of colloidal chemistry.

Lumière¹ likewise subscribes to the colloidal theory of allergy, stating that the phenomenon is one of colloidal flocculation of serum and that the flocculates irritate the endothelium of the cerebral capillaries producing vasodilation, which is transmitted by reflex action to the splanchnic and other capillaries and responsible for the vascular phenomena.

The Production of Passive Anaphylaxis.—An important phase of this subject over which there has been considerable difference of opinion refers to the question whether some time must elapse between the injection of immune serum and anaphylactogen before anaphylaxis is produced, or whether intoxication may follow the simultaneous injection of both antibody and antigen. Thus Gay and Southard, in their early studies, found their recipients first sensitized on the fourteenth day after injection of immune serum; these results, however, may have been due to active sensitization by antigen carried over in the serum. Otto and Friedmann observed shock twenty-four hours after injecting the anaphylactic serum subcutaneously and antigen intraperitoneally. By injecting both serums intravenously and simultaneously, Doerr and Russ finally succeeded in producing acute anaphylaxis and almost immediate death. Weil,² however, believes that the simultaneous injection of antigen and of antiserum into opposite jugular veins in the guinea-pig never produces an anaphylactic reaction, in spite of the use of wide quantitative variations on both substances. On the other hand, if the antigen were injected a few hours after the antiserum, in the same quantitative variations, the reaction occurred regularly. In the rabbit and dog, on the other hand, passive hypersensitiveness occurs almost at the moment of the injection of the antiserum. From this it was concluded that the body cells anchor the antibody during the latent period, and that anaphylaxis is the result of an interaction between the cellular antibodies and the antigen.

It would appear that passive anaphylaxis is not wholly determined by the amounts of antigen or antibody, but by the proportion that exists between the two. For example, Friedmann,³ in his studies on passive homologous anaphylaxis in rabbits, found that, by employing 2.5 c.c. of antiserum with from 2.5 to 0.25 c.c. of antigen, no results were observed, whereas positive reactions were obtained when the amount of antigen was reduced from 0.025 to 0.0025 c.c.

Ordinarily, normal guinea-pigs may be passively sensitized by 0.1 to 0.5 c.c. of serum injected intraperitoneally, and anaphylactized one or two days later by an intravenous injection (0.1 to 0.5 c.c.) of the antigen. The immune serum may be prepared by injecting rabbits with horse-serum,

¹ *Paris méd.*, 1921, 11, 445; *Presse méd.*, 1921, 29, 960.

² *Jour. Med. Research*, 1914, 30, No. 2, 87.

³ *Jahr. u. d. Ergeb. Immunitätsf.*, 1910, vi, 67.

after the methods for the production of precipitins described in Chapter XVII.

DESENSITIZATION AND ANTIANAPHYLAXIS

Desensitization.—The state of allergy to various exciting substances due to active or passive sensitization may be decreased or entirely removed for varying periods of time by certain procedures. For this refractory condition Besredka and Steinhardt¹ originally proposed the terms “antianaphylaxis” and “desensitization”; since then the word “desensitization” has come into general use for designating this particular kind of refractory state or insensibility to further injections that may follow recovery from anaphylactic shock or be induced by one or several administrations of the antigen in subintoxicating amounts.

Desensitization may be accomplished by the administration of non-shocking amounts of the specific sensitinogen (*specific desensitization*) or, to some extent, by the administration of various unrelated substances (*non-specific desensitization*).

Antianaphylaxis.—This is the refractory state due to the presence of sufficient antibodies in the blood to neutralize the antigen and thereby protect the sensitized cells. It is a specific phenomenon and is commonly designated as *antianaphylaxis by antibody protection*.

The phenomenon of desensitization was described by the early investigators in anaphylaxis. For example, Rosenau and Anderson observed that guinea-pigs reinjected with horse-serum before the end of the primary incubation for sensitization, did not become hypersensitive for some time later. They also gave guinea-pigs a series of three or four injections of large amounts of horse-serum at intervals of six days, and finding them refractory to anaphylactic shock, believed that the animals had become immunized against the supposed toxic portion of serum regarded at that time as responsible for the production of acute anaphylactic shock. Similar observations were made by Otto and Besredka. These results are now known to have been due not to immunization against some constituent in the serum, but to desensitization.

Production of Specific Desensitization.—If a guinea-pig that has been actively or passively sensitized be given by subcutaneous or intraperitoneal injection a non-shocking quantity of the respective anaphylactogen, the animal will be found, after an interval of several hours, to have lost its previous hypersensitiveness, so that now it may withstand larger injections in the same manner as a normal guinea-pig. The isolated muscle strips (uteri) from such animals exhibit the same desensitization.

This desensitization is highly specific; if a guinea-pig has been sensitized to two different proteins and recovers from the shock induced by one of them to which it thus becomes desensitized, it still remains sensitive to the other, but to a somewhat lesser degree. This is apparently due in part to non-specific desensitization.

The desensitizing dose of antigen may be given subcutaneously, intraperitoneally, intravenously, or intracerebrally, but the amount injected should be less (five times at least) than that required to produce shock by these different routes of administration. According to Besredka, desensitization of guinea-pigs follows on an average four to five hours after subcutaneous injection, one or two hours after intraperitoneal or intraspinal, and almost instantaneously after intravenous and intracerebral injection. Desensitization may also be effected by oral and rectal administrations of the

¹ Ann. d. l'Inst. Pasteur, 1907, 21, 117, 384.

antigen, but the time required is usually forty-eight to seventy-two hours and the degree of desensitization is less complete. The rectal route is somewhat more rapid than the oral.

A guinea-pig sensitized to horse-serum is usually desensitized partly or completely by the injection of 0.05 c.c. serum subcutaneously, 0.02 c.c. intraperitoneally, and 0.01 c.c. intravenously. A series of injections desensitizes more efficiently than a single injection. For example, the intravenous injection of 0.01 c.c. serum may desensitize against only double the shock-producing dose, but if this injection is followed at intervals of five minutes by the injection of increasing amounts, as 0.02, 0.05, and finally 1 c.c., the animal may be desensitized against twenty or more times the shock-producing dose of antigen.

Besredka has also given the following example of desensitization of guinea-pigs actively sensitized to egg-albumen to such degree that after an incubation period of eighteen days the intravenous injection of 0.002 c.c. was fatal within a minute. For desensitization 0.0005 c.c. egg-albumen was given intravenously; two minutes later 0.002 c.c. was injected without symptoms, followed at ten-minute intervals by the injection of 0.02, 0.2, and finally 2 c.c. Within a period of forty-five minutes, therefore, an animal was desensitized to the extent of being able to survive one thousand times the calculated fatal amount of this substance.

Weil¹ has found that the degree of desensitization does not bear a quantitative relationship to the amount of antigen injected. The antibody measured in terms of precipitin unites with its antigen in the test-tube in constant proportions to form precipitate, but when attached to the cells it appears to combine with antigen in varying proportions so that antigen injected into guinea-pigs passively sensitized "does not depress the reactivity of cellular antibody in regular quantitative ratios." Coca² has suggested that the antibodies may display varying combining power for antigen so that the partially desensitizing injections have the effect of neutralizing only or chiefly the antibodies of the greater avidity.

Another form of transitory desensitization or, rather, unusual delay in development of active sensitization, is observed when guinea-pigs are given large sensitizing injections, as 2 c.c. of horse-serum on each of three successive days. When given the test injection after the usual period of incubation of eight to twelve days they may be found refractory, although the blood does not contain sufficient antibodies to explain the results on the basis of antianaphylaxis due to antibody protection. Weil has explained this on the basis of coexistence of antigen and antibody in the cells, but a simpler explanation is on the basis of the persistence of antigen in the blood over a long period of time which gradually unites with the cellular antibodies as they are formed, and thereby desensitize in a constant and mild manner until the excess of antigen has been consumed in this manner.

The subject of desensitization is of great importance in connection with serum therapy of man and the lower animals; also in relation to the treatment of hay-fever, drug, and bacterial allergies. For this reason it will be considered in greater detail in relation to these subjects in a following chapter.

Mechanism of Specific Desensitization.—Desensitization is commonly regarded as due to the specific neutralization, exhaustion or saturation of the sensitizin or allergic antibodies situated in the cells of the sensitized tissues. Apparently when the sensitinogen is introduced *slowly* and in the

¹ Jour. Immunology, 1917, 2, 469.

² Tice's Practice of Medicine, 1920, 140.

first dose in less than the shock-producing amount, the effects of union with the sensitized cells are not injurious or of minor importance; on the contrary, shock is produced by the *sudden* access of sensitinogen to the sensitized cells. Desensitization may be a process of restoration of colloidal equilibrium within sensitized cells. The colloidal nature of phenomenon is also suggested by non-specific desensitization.

Non-specific Desensitization.—Transitory desensitization of partial degree of guinea-pigs can sometimes be effected by the intravenous injection of numerous substances a short time previous to the test injection. In this connection sodium chlorid and other inorganic salts, peptone, trypsin, urine, foreign serum, or other protein substances, have been employed.

Biedl and Kraus¹ have asserted that animals sensitized to sera can be desensitized with such substances as peptone, and the oral administration of this substance has been advised by others for desensitization in food allergy. Kopaczewski and Vohram² found that sodium oleate reduced anaphylactic shock, which they attributed to a lowering of surface tension of the blood. Sodium chlorid has received special attention in this connection; Richet³ ascribes its effects to an influence upon nerve-cells, while other investigators attribute its inhibitory effect to a modification of the colloidal state of fluids and cells.

In relation to non-specific desensitization of serum anaphylaxis, the injection of beef-serum has lately commanded considerable attention. Penna⁴ has observed in the Argentina that the administration of beef-serum in the treatment of anthrax has rarely produced serum sickness. Pfeiffer and Mita⁵ have protected guinea-pigs sensitized to horse-serum against anaphylactic shock by giving previous injections of beef-serum; Penna, Cuenca, and Kraus⁶ have prevented serum sickness in man by injections of beef-serum.

Non-specific desensitization is not quite the same as antisensitization; for example, in the experiments of Julian Lewis⁷ sensitization may be prevented when horse-serum is first mixed with dog-serum before injection into the lower animals. According to this author, as much as 0.1 c.c. horse-serum in 10 c.c. of dog-serum would not sensitize, even though 0.000,001 c.c. of horse-serum alone produced a high degree of sensitization. Other sera (human, cat, and beef) added to horse-serum had a similar effect.

Karsner and Ecker⁸ have recently reported an extensive series of experiments upon guinea-pigs actively sensitized with horse-, human, and beef-sera; the sera of the horse, beef, goat, swine, dog, cat, rabbit, and human were employed for non-specific desensitization. The authors found that guinea-pigs sensitized to serum may be desensitized to a certain degree by injections of heterologous sera, but never as completely as when homologous serum was employed. They also found that heterologous desensitization was best accomplished by giving the serum intravenously, as has been generally found true in specific desensitization with homologous sera. In general, non-specific desensitization with heterologous sera was of shorter duration than observed with specific desensitization.

This non-specific refractory state is not absolute; shock in full measure

¹ Wien. klin. Wchn., 1909, 22, 363.

² Compt. rend. Acad. d. Sci., 1919, 169, 250.

³ Compt. rend. Acad. d. Sci., 1919, 169, 9.

⁴ Jour. Amer. Med. Assoc., 1918, 71, 587.

⁵ Ztschr. f. Immunitätsf., 1910, 4, 410.

⁶ Rev. de l'Inst. Bacteriol., 1918, 1, 405.

⁷ Jour. Amer. Med. Assoc., 1921, 76, 1342.

⁸ Jour. Infect. Dis., 1922, 30, 333.

can be elicited by the administration of larger amounts of the specific antigen.

The mechanism is not understood, but, as referred to above, is apparently referable to colloidal phenomena within sensitized cells.

The Mechanism of Antianaphylaxis.—As stated above, antianaphylaxis is applied to the refractory state due to the presence of sufficient sensitizin or antibody in the blood to effectually neutralize small amounts of the antigen before the latter reaches sensitized cells with the production of shock. It is not absolute, since Weil¹ has shown that it can be overcome with relatively large intravenous injections of the antigen; Weil has proposed the phrase "antibody protection" to the phenomenon.

Weil found that if small desensitizing quantities of antigen are injected subcutaneously into passively sensitized guinea-pigs that had been given large protective injections of antiserum, the circulatory antibodies are found to be "neutralized," although the uterine muscle still remains sensitive. Final proof of the correctness of these observations was furnished by Manwaring and Kusama, who found that in lung perfusion experiments conducted with sensitized guinea-pigs that the tissues were protected against reaction by the presence of blood.

The phenomenon of antianaphylaxis in this restricted meaning of the term is probably of rare occurrence in human beings inasmuch as the presence of free sensitizin (antibody) in the blood as determined by the success of passive transfer to the lower animals, is relatively uncommon.

LOCAL ALLERGY

Among the earliest allergic phenomena to be described was the local reaction of edema and necrosis observed by Arthus² in rabbits sensitized to horse-serum following the subcutaneous injection of the antigen. Since then it has become well established that local allergic reactions may occur among human beings not only to serum, but to other sensitinogens including various proteins of animal and vegetable origin as well as drugs and other protein substances.

Nicollé³ and Lewis⁴ have observed the Arthus reaction among guinea-pigs actively sensitized with horse-serum, but similar reactions have not been reported occurring in dogs.

In man local allergic reactions are elicited by the application on or the injection of the sensitinogen into the skin or mucous membranes in individuals hypersensitive to foods, pollens, bacteria, and drugs as well as to horse-serum and to the proteins of the skin and hair of the horse and other animals and the feathers of fowl. Of considerable interest in this connection are the observations of Auer⁵ to the effect that injury to the skin of a sensitized animal at a time when the antigen is present in the blood may result in a deposit of the antigen in the injured tissues by extravasation from the blood, with the production of a local allergic reaction.

Passive Sensitization of the Skin and Mucous Membranes.—Passive sensitization of the skin and underlying tissues has been accomplished by Nicollé, who injected 50 to 60 c.c. of antihorse rabbit-serum into each of 5 normal rabbits and found that twenty-four hours later the subcutaneous injection of horse-serum elicited a local Arthus reaction in some animals so

¹ Jour. Immunol., 1917, 2, 157.

² Compt. rend. Soc. de biol., 1903, lv, 817; 1906, lx, 1143.

³ Ann. d. l'Inst. Pasteur, 1907, 21, 128.

⁴ Jour. Exper. Med., 1908, 10, 1.

⁵ Jour. Exper. Med., 1920, 32, 427.

treated. Attempts to confer passive sensitization of rabbits and guinea-pigs with injections of the sera of human beings hypersensitive to pollens, bacteria, foods, and drugs have generally failed, and for this reason Coca regards the Arthus phenomenon as the only true anaphylactic (antigen-antibody) reaction. As previously stated, this does not appear to be a warrantable conclusion; even the sera of highly sensitized guinea-pigs may contain too few circulating antibodies to passively sensitize other pigs, and the cells of man may readily be sensitized to a protein without there being sufficient free antibodies in the blood to confer passive heterologous sensitization.

Schultz and Larson,¹ for example, claim to have passively sensitized guinea-pigs with the blood of infants with exudative diathesis apparently due to milk allergy, and Ramirez² has recorded a striking example of passive transfer of allergy to horse protein in a human being. A man who had never had asthma, hay-fever, urticaria, and the like received a transfusion of 600 c.c. of blood from a man with typical horse asthma who gave a cutaneous reaction to horse dandruff in 1 : 50,000 dilution. Two weeks later the transfused patient went for a carriage ride and within five minutes had a typical attack of asthma, and a skin test yielded a positive reaction to horse dandruff diluted 1 : 20,000, but not to other proteins.

Tuberculin hypersensitiveness apparently has been passively transferred from tuberculous to normal animals by Helmholtz³ and Austrian,⁴ but against these positive results is a long list of negative results.

Active Sensitization of the Skin and Mucous Membranes.—Sensitization of the skin and mucous membranes develops more actively among human beings than among the lower animals. However, in serum allergy the skin and mucous membranes may not demonstrate sensitiveness when tested by the usual methods, whereas the administration of serum may elicit a general allergic reaction.

During active immunization of man and the lower animals with bacterial vaccines skin hypersensitiveness sometimes develops as demonstrated by unusually severe local reactions following subcutaneous injections.

Hypersensitiveness in man and the lower animals to tuberculin, however, apparently only occurs in active tuberculosis, as shown so clearly in the valuable researches of Kraus.⁵ While it is easily possible to sensitize guinea-pigs to tuberculoprotein so that they will yield general or systemic allergic reactions, such animals do not display hypersensitiveness of the skin and mucous membranes to either tuberculoprotein or tuberculin. Skin hypersensitiveness to tuberculin is apparently engendered only by producing an actual tuberculous infection or by the injection of animals with an emulsion of tuberculous tissue as described by Bail⁶ and confirmed by Onaka.⁷

On the other hand, acquired hypersensitiveness of human beings to tuberculin has been described, the allergy developing in both tuberculous and non-tuberculous individuals after repeated administrations and characterized by various skin eruptions, sneezing, lacrimation, or general symptoms.

According to Fleischner, Meyer, and Shaw⁸ the immunization with dead typhoid and contagious abortion bacilli may produce systemic allergic sen-

¹ Arch. Pediat., 1918, 35, 705.

² Jour. Amer. Med. Assoc., 1919, lxxiii, 984.

³ Ztschr. f. Immunitätsf., 1909, 3, 371.

⁴ Jour. Exper. Med., 1912, 15, 149.

⁵ Amer. Rev. Tuberc., 1917, 1, 65.

⁶ Ztschr. f. Immunitätsf., 1909, 4, 470; 1912, 12, 451.

⁷ Ztschr. f. Immunitätsf., 1910, 7, 507.

⁸ Amer. Jour. Dis. Child., 1919, 18, 577.

sitization, but not hypersensitiveness of the skin, the latter being engendered only by living bacilli as in induced skin hypersensitiveness to tuberculin. As previously discussed under sensitins, these investigations suggest that bacterial sensitins of this kind are products of living bacteria and not merely the protein of the bacterial cells.

The administration of horse-serum may result in the production of skin hypersensitiveness, but the most striking examples of this condition are met with among horse asthmatics, who develop extreme hypersensitiveness in unknown ways.

Pollen hypersensitiveness is apparently acquired by inhalation of the pollen, but the subcutaneous injection of pollens does not appear to actively sensitize, according to Cooke, Flood, and Coca.¹ According to these investigators sensitization to pollens and foods are established spontaneously and never by immunologic processes.

The Mechanism of the Skin Allergic Reaction.—Very little is definitely known of this subject; by analogy it is regarded as fundamentally the same as the general or systemic reaction.

It is to be assumed that the cells of the part are sensitized and contain antibodies, and this refers not only to the smooth muscles of the blood-vessels in the subcutaneous and submucous tissues, but to the connective tissue and even the epithelial cells of the parts yielding a reaction. Mere installation of the antigen into the conjunctival sac or injection into epidermis may elicit a reaction and thereby demonstrate sensitization of the cells with which the antigen is brought into relation.

Positive reactions have been explained by the humoralists on the basis of the production of a poison (anaphylatoxin) either by the interaction of antigen, specific amboceptors and complement, or by the digestive activity of proteolytic ferments. Both of these hypotheses have been previously discussed and found inadequate. The writer, who now subscribes to the cellular theory of allergy, is of the opinion that the specific allergic skin reaction is due to an intracellular disturbance of colloidal equilibrium in the same manner as the general allergic reaction.

However, the local lesion may not be entirely one of allergy, and especially if the antigen has been injected intracutaneously or subcutaneously. Under these circumstances there is no reason to doubt that cellular proteolytic enzymes may bring about the digestion of a portion of the injected antigen in a purely non-specific manner, and that the resulting products may aid in the production of inflammatory changes.

Furthermore, the lesion may be due in part to mechanical irritation by the injected substances and particularly the preservative, as well as to the trauma induced by the needle. For this reason a local skin reaction may be due to (a) a true allergic reaction plus (b) a non-specific protein digestion, and (c) trauma.

The Symptoms and Lesions of Local Allergy.—The symptoms are largely due to vascular changes. Prominent symptoms are erythema and edema (urticaria). In hay-fever the nasal mucosa is markedly hyperemic and edematous, and these changes may involve the bronchial mucosa with the production of asthma.

The same changes occur in the skin reactions to pollens, tuberculin, foods, drugs, etc. In extreme hypersensitiveness, as in horse asthma or pollen asthma, the mere application of the sensitizer or exciting agent to an abrasion of the skin is followed in a few to fifteen minutes by the development of a wheal or urticarial lesion accompanied by a burning sensation.

¹ Jour. Immunology, 1917, 2, 217.

It would appear that these changes are due primarily to vasomotor paralysis, that is, extreme relaxation of the vessel walls which may follow a primary contraction of the smooth muscle of the involved parts. This relaxation is quickly followed by extravasations of serum and leukocytes, and especially of eosinophils. Bandler and Kreibich¹ and Daels² have described histologic changes of tuberculosis in local tuberculin reactions; the writer has not found any changes of this kind, however, in excised lesions sixty hours after the cutaneous application of tuberculin.

A more complete description of these lesions will be given in the succeeding chapter.

SPECIFICITY OF ALLERGY

Extensive investigations on anaphylaxis of guinea-pigs sensitized with various proteins of animal and vegetable origin have indicated that anaphylaxis is a highly specific phenomenon; the degree of specificity compares favorably with that observed with the precipitin and complement-fixation reactions.

Drug allergies are known to be highly specific and the same is apparently true of allergies in man to bacteria and their products, serum, pollens, etc. In drug allergies the specificity is often referable to a certain element or chemical group. For example, in allergy or hypersensitiveness to mercurial compounds the active portion is the element mercury; in the allergy to iodoform, the methyl group rather than the iodid or iodine is apparently the allergenic group. Iodine hypersensitiveness, however, does exist as shown by allergy to the iodids of certain metals and not to their chlorids.

It is true that group reactions are sometimes observed, but, as stated by Wells, this is due to the fact that the substances (serum, egg-albumen, milk, etc.) commonly employed as antigens are very complex and contain a number of sensitizing substances and especially when administered in large amounts. Experiments conducted with isolated proteins purified as much as possible, and especially with the proteins obtainable from seeds and nuts, many of which are crystallizable and soluble in alcohol, have indicated striking degrees of specificity, as tested by active sensitization and desensitization.

The *in vitro* anaphylactic reaction conducted with strips of uteri from sensitized young virgin guinea-pigs after the Schultz-Dale method, frequently exhibits sharper and clearer evidences of specificity than reactions produced in the living animal. According to Doerr the subcutaneous injection of antigen, while eliciting a slower response, may prove more specific than reactions produced by intravenous injections.

Working with purified vegetable proteins Wells and Osborne³ found that *the chemical composition and not biologic origin determines specificity*. This is a very important conclusion and of fundamental importance. In other words, chemically similar proteins from seeds of different genera were found to react anaphylactically with one another, while chemically dissimilar proteins from the same seed failed to do so in many cases. This led to a second conclusion by these investigators, namely, that in the protein molecule there are only certain groups essentially concerned in the specific nature of the anaphylaxis reaction.

As to what groupings in the protein molecule determine the specificity,

¹ Deutsch. med. Wchn., 1907, 1629.

² Med. Klin., 1908, 58.

³ Jour. Infect. Dis., 1916, 19, 183.

little is known. On the basis of precipitin tests Obermayer and Pick believed that specificity was determined by the aromatic radicals, but this could not be corroborated by Wells in anaphylaxis experiments. When two protein derivatives (halogenized, azotized, etc.) react with each other, the position in the molecule of the substituted radicals is apparently identical or closely related and the cross reactions dependent on chemical relationships.

While the anaphylactic reaction is one of the most specific of biologic phenomena, nevertheless group reactions occur as in the precipitin and complement-fixation reactions. At one time it was hoped that the reaction would differentiate one organ or tissue of an animal from another organ of the same animal. Ranzi¹ succeeded in sensitizing guinea-pigs with extracts of various tissues, but did not observe any evidences of organ specificity; similar conclusions were arrived at by Minet and Bruyant.² With the proteins of the lens of the eye, however, organ specificity has been observed. Kopsenberg³ rendered guinea-pigs anaphylactic by giving large sensitizing injections of guinea-pig lens; with the lens substance of other animals sensitization resulted with smaller amounts. Anaphylactic reactions were produced by injections of lens substance from any species, but not by the serum. In other words, anaphylaxis to lens protein is organ specific, but not species specific.

The specificity of bacterial anaphylaxis has been the subject of considerable investigation and controversy. Kraus and Doerr found it rigidly specific; according to them guinea-pigs sensitized with typhoid bacilli respond only to a second injection of typhoid and remain unaffected by injections of paratyphoid bacilli. Indeed, Kraus and Admiradzibi⁴ later claimed that the anaphylactic reaction differentiated among strains of the same bacterium. Delarroe⁵ was not able to confirm this degree of specificity and subsequent researches by Studinski⁶ and Nefedoff⁷ likewise show that the specificity of bacterial anaphylaxis while high, is not as rigid as believed by Kraus.

Specific and Non-specific Focal Reactions.—The reaction of hyperemia, edema, pain, etc., developing around foci of tuberculosis after the injection of a sufficient amount of tuberculin and the "lighting up" of abscesses and other lesions after the injection of vaccines, are known as *focal reactions*, and in tuberculosis especially possess valuable diagnostic significance. These reactions are commonly regarded as specific allergic reactions due to the union of antigen and allergic antibody in or upon the sensitized cells.

It is well known, however, that the injection of other non-specific agents, as typhoid vaccine and proteoses intravenously or milk intramuscularly, may elicit similar reactions regarded as due to increased cellular activity (the plasma activation of Weichardt) and increased permeability of lymphatic and capillary channels with an outpouring of lymph, plasma, leukocytes, enzymes, etc. The enzymes doubtless aid in the production of protein poisons which, being absorbed from the foci along with other poisons, contribute to the production of a general or constitutional reaction of fever, tachycardia, etc. This dual character of the focal reaction will be discussed in more detail in Chapter XXXIX.

¹ Ztschr. f. Immunitätsf., 1909, 2, 12.

² Compt. rend. Soc. de biol., 1911, lxxi, 166.

³ Ztschr. f. Immunitätsf., 1912, 15, 518.

⁴ Ztschr. f. Immunitätsf., 1910, 4, 607.

⁵ Compt. rend. Soc. de biol., 1909, lxvi, 207, 252, 248, 389.

⁶ Compt. rend. Soc. de biol., 1911, lxx, 173.

⁷ Compt. rend. Soc. de biol., 1913, lxxiv, 672.

PRACTICAL DIAGNOSTIC APPLICATIONS

Differentiation of Proteins by the Anaphylaxis Reaction.—The specificity of the anaphylactic reaction has led to many attempts to apply it to the differentiation of proteins in a practical way and especially in the differentiation of blood-stains and meats for medicolegal purposes.

Thomsen¹ has used the method for the differentiation of blood-stains, as likewise Uhlenhuth² and others. Guinea-pigs are sensitized by injections of extracts of the stain and after a suitable period of incubation the animals are reinjected with the sera of different animals to determine to which sensitization has been effected. I have conducted these tests by sensitizing a series of guinea-pigs weighing approximately 300 gm. with intraperitoneal injections of 5 c.c. of approximately 1 : 100 dilutions of the blood-stain. After an interval of twenty-one days the animals were given human serum intravenously in dose of 0.1 c.c. and control animals the same amount of serum from other sources, as the dog, chicken, horse, sheep, and ox. For the detection of human blood this test is fairly satisfactory and in medicolegal work may be employed in conjunction with the precipitin and complement-fixation tests when sufficient material is available. Uhlenhuth believes that the test has value only when the precipitable properties of the unknown protein have been lost by preservation or decomposition.

The test has also been employed for the detection of meat adulteration and especially for the detection of cooked dog and other meats in sausage. Guinea-pigs are sensitized with saline extracts of the material and the test injections conducted with the corresponding sera by intravenous administration.

Pfeiffer and Finsterer³ have attempted to employ the reaction for the diagnosis of cancer; guinea-pigs were injected with the sera of patients (passive sensitization) and injected forty-eight hours later with juices expressed from tumors. They believed that in some instances positive and specific reactions were observed (mainly temperature changes), but Ranzi⁴ and others have failed to confirm these observations.

The anaphylaxis reaction has also been applied for the differentiation of bacteria, but the uncertainties of sensitization and other practical difficulties limit the usefulness of this method, and the results so far have been generally less satisfactory than those observed with the agglutination and complement-fixation reactions.

Probably the employment of the Schultz-Dale *in vitro* anaphylaxis reaction conducted with excised uteri of young virgin guinea-pigs sensitized with these protein substances, and especially extracts of blood-stains, will yield better results than those observed with living animals.

Diagnosis of Disease.—Anaphylactic skin reactions have been widely employed for the diagnosis of tuberculosis, syphilis, glanders, typhoid fever, etc.; these will be described in a subsequent chapter.

¹ Ztschr. f. Immunitätsf., 1908-09, 1, 741.

² Ztschr. f. Immunitätsf., 1908-09, 1, 770; referate, 1909, 1, 525.

³ Wien. klin. Wchn., 1909, No. 28.

⁴ Ztschr. f. Immunitätsf., 1909, 2, 12.

PART IV

CHAPTER XXIX

ALLERGY IN RELATION TO INFECTION AND IMMUNITY

BACTERIAL ANAPHYLAXIS

In the preceding chapter mention was made that the proteins of micro-parasites embracing both the bacteria and protozoa may bring about active sensitization of the cells of man and the lower animals. By reason of the importance of this subject to infection and immunity, it is worthy of special discussion, although all that has already been discussed of anaphylaxis to proteins in general, may be broadly applied to bacterial anaphylaxis.

Active Sensitization by Bacteria and Protozoa.—Rosenau and Anderson,¹ in one of their earliest investigations in anaphylaxis, conceived that bacterial proteins may sensitize body cells, and they succeeded in sensitizing guinea-pigs with extracts of colon, typhoid, subtilis, tubercle, and anthrax bacilli as well as with some yeasts. Relatively large amounts of these extracts were required for sensitization and for the production of anaphylactic shock, but they showed that bacterial anaphylaxis and desensitization could be accomplished.

These results were soon corroborated by Kraus and Doerr,² Holobut,³ and others. Delanöe⁴ questioned the specificity of bacterial anaphylactic reactions, but, as stated in the preceding chapter, it is now known that bacterial anaphylaxis possesses the same degree of specificity as characterizes anaphylaxis in general.

For the active sensitization of guinea-pigs a single injection of the bacterial substance may fail, and especially so, since the administration of a large amount may kill the animals by reason of toxic effects. Better results are usually obtained by suspending a loopful of the bacteria in 2 c.c. of salt solution, heating at 56° C. for about thirty minutes, and injecting 1 c.c. subcutaneously or intraperitoneally daily for at least ten days. The test injection may be made about three weeks after the last dose, should be given intravenously and in rather large amount, as 2 c.c. of a heavy suspension. A non-sensitized control animal should always be included to make sure that the test dose is without anaphylactoid effects.

The proteins of practically all bacterial cells may act as sensitinogens for guinea-pigs and presumably for the body cells of man. Owing, however, to the physical state of these anaphylactogens, being usually particulate bodies in suspension rather than solutions, sensitization is both tardier and less complete than is usually observed with the soluble proteins.

Apparently sensitization of man is sometimes effected by repeated subcutaneous injections of bacterial suspensions, and not infrequently the unusually severe local reactions sometimes following these administrations are in part anaphylactic reactions.

¹ U. S. Public Health and M. H. S. Hyg. Lab. Bull., 1907, No. 36.

² Wien. klin. Wchn., 1908, No. 28.

³ Ztschr. f. Immunitätsf., 1909, 3.

⁴ Compt. rend. Soc. de biol., 1909, 66, 207, 252, 348, 389.

Sensitization of guinea-pigs has also been accomplished with extracts of pathogenic and non-pathogenic yeasts and fungi, as well as with such protozoa as the cyst fluid of *Tænia echinococcus* and extracts of other helminths.

Active Sensitization in Infectious Diseases.—Unquestionably active sensitization of the body cells may occur during the course of some bacterial and protozoan diseases and especially in those of a *prolonged* or *chronic* character. A striking example of bacterial sensitization of man is afforded by numerous cases of bronchial asthma, apparently due to sensitization by bacteria in the inflamed mucosa of the respiratory tract.

This sensitization is apparently effected by both the proteins of the bacterial cells and possibly by excretory products. *But the mechanism of sensitization of the skin in tuberculosis and possibly in other infectious diseases is obscure and unique and apparently engendered by the presence and activities of the living bacteria in the tissues.* For example, sensitization and skin reactions to tuberculin can be acquired only by the stimulation afforded by foci of tuberculosis as shown so conclusively by Kraus¹; numerous attempts to sensitize animals with Koch's old tuberculin and similar products have failed. Apparently the sensitizing substance is similar to the tuberculin produced in cultures, but the latter does not serve for sensitization while that produced in the tissues sensitizes the skin and tuberculous tissues to an exquisite degree. The sensitizing agent is certainly in the tissues of the tubercle and has been successively transmitted by Bail to normal animals by injections of suspensions of tuberculous tissue. Fleischner, Meyer, and Shaw² have corroborated these observations on tuberculin hypersensitiveness of the skin, and find that it is equally true for typhoid bacilli and *Bacillus abortus*. Similar results have been observed by Zinsser and Parker³ in experiments carried out with tubercle bacilli, pneumococci, staphylococci, influenza bacilli, and typhoid bacilli. *Judging by the local or skin reactions of hypersensitiveness, sensitization in disease may, therefore, be produced by the bacterial proteins, on the one hand, and by a product of the living bacteria in the tissues on the other.* Since the tuberculin skin reaction cannot be elicited by immunization of healthy animals with tuberculin, the phenomenon is not considered as an allergic reaction by many observers, but I prefer to so list it in the present state of our knowledge pending further investigations, believing that in tuberculosis and probably in other bacterial infections as well, we are dealing with a peculiar kind of active sensitization by bacterial substances produced only in the living animal during the course of disease in addition to sensitization to the bacterial proteins (a true anaphylaxis) in at least some instances.

Whether anaphylactic sensitization occurs in all infectious diseases of bacterial, mycotic, and protozoan origin and in infestments with helminths, cannot be stated. Our data is almost solely based upon the results of skin tests. Studies on the comparative sensitizing rates of the proteins from different microparasites do not appear to have been made. Based upon our general knowledge of sensitization, one may surmise that the sensitizing properties are equal when the respective proteins are rendered soluble in the body fluids, but that the rate, degree, and amount of solution in different diseases varies widely and that in diseases of short duration may not occur at all or to an insufficient degree. But that active sensitization may occur in those infectious diseases where the conditions of time and adequate amounts of sensitizing substances are met, would appear to be the case and

¹ Amer. Rev. Tuberculosis, 1917, 1, 65.

² Amer. Jour. Dis. Child., 1919, 18, 577.

³ Jour. Exper. Med., 1921, 34, 495; *ibid.*, 1923, 37, 275.

worthy of the closest study in relation to the course and treatment of infectious diseases.

Active Sensitization by Toxins; Toxin Hypersensitiveness.—Among the earliest observations on hypersensitiveness are those of von Behring,¹ who found that animals once injected with non-lethal amounts of both diphtheria and tetanus toxins occasionally became more sensitive to them subsequently than normal animals. These results were particularly puzzling because increased tolerance rather than intolerance was expected owing to the production of antitoxins. Repeated injections of toxin may induce such a state of hypersensitiveness than even 1/700 of the usual non-lethal dose may prove fatal.

The condition is sometimes encountered in horses undergoing toxin immunization, but is most easily and regularly induced in guinea-pigs by repeated injections at short intervals. Lowenstein² claims to have produced hypersensitiveness to tetanus toxin by a single injection and with an incubation period of at least twenty days; according to Kretz³ repeated injections of diphtheria toxin and ricin are required.

Various explanations have been offered. Some investigators have theorized that it is due to the presence of an excess of toxin receptors attached to the body cells (sessile receptors) possessing an increased affinity for the toxins, and that as a result of this the toxins when injected are rapidly anchored with disastrous effects. However, this explanation appears insufficient because reactions may occur at a time when the blood contains specific antitoxins and it is difficult to understand how the injected toxin escapes sufficient neutralization by these to protect the cells even though these may have a greater affinity for the toxin than the free receptors (antitoxin).

That the condition is not a mere summation of toxic effects is shown by Loewi and Meyer,⁴ who found with spaced injections that the total amount injected was less than a single minimal lethal dose. According to these observers hypersensitiveness to tetanus toxin is due to changes in the ganglion cells of the spinal cord because of traces of toxin retained in them.

Friedberger, Mita, and Kumagai⁵ have sought to explain the phenomenon on the basis of "anaphylatoxin" production by specific amboceptors and complement. They succeeded in rendering normal guinea-pig serum toxic by the addition of minute amounts of dried toxin, but these results are analogous to those observed in the treatment of serum with agar, kaolin, and the like, and, as discussed in the preceding chapter, are insufficient for explaining anaphylaxis.

While Friedberger's theory is not acceptable, yet in the writer's opinion toxin hypersensitiveness is an allergic phenomenon and probably anaphylactic. The exact chemical constitutions of tetanus and diphtheria toxins are unknown, but available data indicates that they are protein substances; even if proteoses, it is possible that they may act as sensitinogens. While the symptoms of tetanus toxin hypersensitiveness are said to be largely those of tetanus, it is not at all improbable that the ganglion cells of the central nervous system possessing a known affinity for this toxin are likewise with other cells acutely sensitized. Under these conditions they would participate in the anaphylactic shock, and if so, symptoms of tetanus may be expected as a result of overstimulation of their physiologic functions.

¹ Deutsch. med. Wchn., 1893, xlviii, 1253.

² Ztschr. f. exper. Path. u. Therap., 1914, 15, 279.

³ Ztschr. f. Heilk., 1902.

⁴ Ztschr. f. exper. Path. u. Pharmacol. Suppl. Bd., 1908, 355.

⁵ Ztschr. f. Immunitätsf., 1913, 17, 506.

While it is true that precipitins have not been found in the blood of hypersensitive animals, and that all attempts to passively transfer the condition to normal animals have failed, these observations cannot carry much weight against the probable anaphylactic nature of toxin hypersensitiveness for reasons discussed in the preceding chapter. I regard toxin hypersensitiveness as an allergic phenomenon due to disturbances of intracellular colloidal equilibrium of the same nature and induced in the same manner as allergy to other substances.

Relation of Bacterial Anaphylaxis to Disease.—Since active sensitization may occur during some of the infectious diseases caused by bacteria, fungi (as ringworm, favus, sporotrichosis, actinomycosis, etc.), and protozoa, a list of these diseases being given in the succeeding chapter, the question naturally arises regarding the relation of anaphylaxis to the cause and course of infectious diseases.

According to the humoral theories of anaphylaxis the relation is a very intimate one. It will be remembered that these theories are divisible into three kinds, briefly as follows: (a) That anaphylaxis is due to the production of poisons by the action of specific amboceptors (proteolysins) and complement upon the antigen; (b) that anaphylaxis is due to the production of a poison from the antigen by the digestive activity of specific ferments, and (c) that anaphylaxis is due to the production of a poison by the digestion of the serum by serum ferments or proteases. While the theories differ in regard to the mechanism of the production of a protein poison, all are based upon the assumption that such a poison (anaphylatoxin, serotoxin) is produced and primarily responsible for the phenomena of anaphylaxis. The writer has been until the last few years an adherent of the humoral theories, but cannot subscribe to them now in view of the mass of excellent experimental data accumulated by Weil, Doerr, Coca, and others in favor of the cellular theory.

I am convinced that poisonous substances may be produced *in vitro* and presumably in the living animal, by the interaction of antigen, specific antibody, and complement. Even though such poisons have not been conclusively shown in the blood during anaphylaxis, they may develop during the course of disease and doubtless contribute to the production of lesions and symptoms, but this is not anaphylaxis. The same may be admitted for the production of such poisons by the cellular and plasma proteases. The writer cannot subscribe to the broad and sweeping views of Vaughan, Friedberger, von Pirquet, and others that such poisons are the cause of anaphylaxis, although it is freely admitted that they are probably produced during disease and not only in those diseases caused by microparasites, but in non-infectious diseases as well where cellular death is prominent. Doubtless they exert an important rôle in the production of symptoms and lesions, and for this reason I have included them in the chapters dealing with infection and the production of infectious diseases.

This leaves for consideration the probable rôle of anaphylaxis in disease in a strict sense and in terms of the cellular colloidal nature of the phenomenon discussed in the preceding chapter.

The symptoms of allergy in man may be so diverse that many of the symptoms ordinarily attributed to bacterial toxins may be expressions of anaphylaxis. The essential anaphylactic lesion is apparently contraction of smooth muscle followed by relaxation. For this reason dilatation of vessels accompanied by serous exudation are characteristic changes of the allergic reaction in human beings. These effects are apparently responsible for the skin lesions and particularly of the urticaria in general allergy; swelling of

the lips, tongue and diarrhea in alimentary allergy and swelling and congestion of the mucosa of the respiratory tract in pollen and bacterial allergy. It may well be that the exanthemata of some of the acute infectious diseases are anaphylactic reactions, as so considered and grouped by von Pirquet.

The allergic reaction generally first occurs at the place of introduction of the exciting substance. For example, the inhalation of pollen excites a local reaction of the nasal mucosa and if deeply inhaled may result in lesions in the trachea and bronchi. In food allergy, the lips and tongue may show the first vasomotor changes upon contact with the exciting substance. In bacterial anaphylactic asthma the changes are primarily in the bronchi. It is possible that the ordinary "common cold" is an anaphylactic reaction, the lesions occurring primarily in the nasal mucosa upon inhalation of the exciting agent.

Probably a close analysis of many of the infectious diseases would show that some of the lesions and symptoms could reasonably be explained on the basis of anaphylaxis in a strict sense and as a cellular phenomenon without confusing the subject with the production of protein poisons. Doubtless the factors of antianaphylaxis by antibody protection, the effects of co-existence of antigen and sensitizin or allergic antibody in the cells and blood and desensitization would require consideration as modifying factors. At least the essential facts are apparent, namely, that the microparasites of disease contain proteins which may sensitize anaphylactically and some may produce a more active sensitinogen in the tissues. Under proper conditions it is reasonable to assume that anaphylactic shock or shocks may occur during the course of a given disease and the effects of these may contribute to the lesions and symptomatology in no small degree.

The Relation of Anaphylaxis to Immunity.—Many adherents of the humoral theory of anaphylaxis believe that the same antibody or ferment mechanism capable of digesting the proteins of microparasites with the production of the poisons regarded as responsible for anaphylaxis, may attack and "split" the proteins of the *living* parasites, bringing about the destruction of the latter and thereby indicating a very close relation of anaphylaxis to the phenomena of immunity and recovery from infectious disease. In other words, according to these views, either the anaphylactic antibody may be itself destructive of living microparasites or its production is accompanied by other antibodies having these properties.

Romer¹ and Sata,² in experiments among cattle with *Bacillus tuberculosis*, reached the conclusion that a state of hypersensitiveness meant a certain degree of resistance, while Krause³ and Austrian⁴ have expressed the opinion, based upon experiments, that sensitization of non-tuberculous animals with tubercle protein does not raise their resistance to experimental tuberculosis infection, and, indeed, may lower it.

More recently Gay and Force⁵ have greatly renewed interest in this subject by advocating the anaphylactic skin test as a means of determining defensive activity following typhoid fever or active immunization by means of vaccines. Their first work was conducted with a "typhoidin" prepared in the same manner as Koch's old tuberculin by cutaneous inoculation. Later Gay and Claypole⁶ prepared typhoidin by precipitating the solution

¹ Beitr. z. Klinik. d. Tuberk., 1908, xi, 79.

² Ztschr. f. Tuberk., 1911, xviii, 1.

³ Jour. Med. Research, 1911, xxiv, 361; *ibid.*, 1916, 35, 1, 35.

⁴ Bull. Johns Hopkins Hosp., 1913, xxiv, 11.

⁵ Archiv. Int. Med., 1914, xiii, 471.

⁶ Archiv. Int. Med., 1914, xiv, 671.

with alcohol, washing the precipitate with alcohol and ether, drying in a vacuum, and suspending the resulting powder in phenolized normal salt solution, which was injected intracutaneously and applied cutaneously, a control powder being prepared from broth and used in the same manner. With this skin test Gay and his associates have studied the relative value of various vaccines and regard the anaphylactic reaction as indicative of a state of immunity. Nichols¹ has questioned the value of the anaphylactic skin test as an index of immunity and regards the typhoidin reaction as indicating nothing more than sensitization to typhoid protein, which is apparently less lasting and less specific than the true immunity to this infection.

The experiments of my associates and myself² in this field have been largely tests *in vitro* for various antibodies, as agglutinins, bacteriolysins, and complement-fixing substances in the fresh sterile blood sera of persons and lower animals hypersensitive to various proteins (typhoid, syphilis, diphtheria, and canine distemper), and have shown that the state of hypersensitiveness to a particular bacterial protein bears no relation to the presence or absence of demonstrable amounts of these antibodies. The experiments of Meyer³ have corroborated our results and conclusions, and have furthermore shown that a positive typhoidin skin reaction in a rabbit does not indicate the presence of resistance to an infection with *Bacillus typhosus*.

The sum total of these studies indicate that although antibodies that may be regarded as possessing protective and curative properties toward a certain bacterium *may* be present in the body fluids of persons and animals hypersensitive to this particular protein, the condition of hypersensitiveness in itself is no direct evidence of their presence of resistance to a particular infection, although these antibodies are most likely to be present in the body fluids of those persons who are hypersensitive. The positive anaphylactic skin test is, therefore, evidence of infection or sensitization to a particular protein probably without bearing any direct relation to resistance to infection or reinfection.

This still leaves open the question, however, whether the anaphylactic antibody is capable of attacking and destroying *living* microparasites. It is well known that while a single or a few injections of bacteria may sensitize the cells of an animal that additional injections may render it anaphylactically immune. For this reason sensitization probably always occurs as an early step in immunization. The immunity apparently depends upon antibodies in the blood which neutralize the antigen before it reaches the cells. Manwaring and Kusama, for example, found that the lungs of a sensitized guinea-pig were protected against anaphylactic shock as long as they contained blood, but when thoroughly washed out they reacted upon the introduction of the antigen. This immunity of the cells is an example of anti-anaphylaxis by antibody protection; it is an immunity of the sensitized cells against the introduced protein. The question naturally arises: Are these antibodies responsible for immunity or protection against anaphylactic shock able to destroy or neutralize a living antigen in the form of a virulent microparasite or is this the function of the leukocytes and other defensive agencies, the anaphylactic antibody being powerless to kill, but capable of protecting the sensitized cells against the dead proteins?

In a strict sense, therefore, the relation of anaphylaxis to the questions

¹ Jour. Exper. Med., 1915, xxii, 780.

² Jour. Immunology, 1916, 1, 409; *ibid.*, 1916, 1, 429; *ibid.*, 1916, 1, 443; *ibid.*, 1916, 1, 571.

³ Jour. Infect. Dis., 1917, xx, 424.

of immunity and recovery from disease resolves itself into whether or not the sessile (cellular) or free anaphylactic antibody is capable of destroying living microparasites. Available data upon which to express a definite opinion is too meager, but the writer does not believe that such is the case. However, if the anaphylactic antibody is bactericidal, anaphylaxis and immunity bear a very intimate relationship.

Curative Influence of Anaphylactic Shock.—Since protection of sensitized cells against anaphylactic shock appears to depend in part upon whether or not sufficient anaphylactic antibody is present in the blood for the neutralization of the antigen, it is apparent that shock or shocks may occur during the course of bacterial diseases when the antibody is not present or insufficient. The question then arises: Has anaphylactic shock any beneficial or curative effects?

In all probability the reaction of congestion and exudation about foci of tuberculosis following the subcutaneous injection of a sufficient amount of tuberculin, is an anaphylactic reaction as well as in part a non-specific reaction (see Chapter XLI). Likewise similar reactions about foci of other bacterial infections following the administration of adequate amounts of bacterial vaccines. Are these reactions beneficial? Kraus has evidence that focal tuberculin reactions gradually result in an increase of fibrous tissue about the lesions and this is certainly desirable; it is commonly believed on the basis of clinical experience that focal reactions after the administration of bacterial vaccines are of good prognostic import. It would appear, therefore, that when these reactions are mild and not too frequent that they are beneficial; when too violent they are doubtless very harmful as shown in the early days of tuberculin therapy with excessive doses, the lamentable days of the "tuberculin delirium."

Protein "shock" therapy may be under certain circumstances an anaphylactic phenomenon when the antigen is specific; as ordinarily practised it is apparently not of an anaphylactic nature and will be further discussed in Chapter XXXIX with special reference to its therapeutic effects.

CHAPTER XXX

CLINICAL ALLERGY

Importance and Frequency of Allergy in Man.—While in the early days of investigations in anaphylaxis it was believed that the manifestations in man were confined to those cases developing after the administration of horse-serum antitoxin, it is now known that man is subject to allergy by many different kinds of exciting agents and the subject has come to occupy a very important place in medicine. The manifestations of allergy are likewise many and diverse and in some instances unexplainable lesions and symptoms of disease have been discovered to be allergic in nature. The list of exciting agents embraces not only the sera of the lower animals and especially of the horse, but emanations from the skin, hair, and expired air of many of the lower animals; also different bacteria, fungi, and protozoa as well as pollens and juices of some plants, various foods of animal and vegetable origin, and drugs. Cooke and Vander Veer have estimated that the frequency of human sensitizations *with clinical manifestations* is probably not over 10 per cent.; the results of skin tests, however, show a larger percentage of individuals apparently hypersensitive to some protein or proteins although without symptoms, probably because the proteins as such never reach the sensitized cells.

Natural and Acquired Allergy.—Is the allergic state or hypersensitiveness inheritable, that is, is there a variety of true natural allergy? Otto and Rosenau and Anderson have shown that mother guinea-pigs sensitized to horse-serum may transmit this anaphylaxis passively to their young, but in the latter the state of hypersensitiveness is of short duration and does not usually last more than six weeks. It has never been shown experimentally that a sensitized father guinea-pig transmits a sensitization. In other words, it would appear that a mother may passively transmit a transient hypersensitiveness by passage of the anaphylactic antibody through the placenta, but whether this occurs with human mothers is unknown.

Whether a child may acquire active sensitization before birth is very doubtful; it would appear that placental transmission of the exciting agent resulting in prenatal active sensitization to a given substance is at least rare and unusual, although the occasional occurrence of hypersensitiveness to foods among infants suggests that this may occur.

However, *a tendency to acquire sensitization may be inherited* and, indeed, constitutes a very important phase of human sensitizations, as shown by the investigations of Cooke and Vander Veer with special reference to hay-fever. When both father and mother had hay-fever, the children did not show any symptoms during the first few years constituting evidence against the actual transmission of the sensitization, but the transmission of the tendency to acquire sensitization is shown by the fact that 36.3 per cent. of such children showed symptoms before the fifth year of age. When one parent was hypersensitive about 14.3 per cent. of the group of children showed symptoms before the fifth year, while only 5 per cent. of a group in which the parents were healthy developed symptoms at this period.

These studies have indicated that *allergic individuals do not transmit to their offspring their own sensitizations, but an unusual capacity for acquiring sensitization to a variety of substances*; this tendency is apparently inherited

as a dominant characteristic. The nature of the inherited tendency is unknown; Cooke and Vander Veer have not found these individuals any easier to sensitize artificially than average individuals, so that the mechanism is dependent upon some other factor than the mere administration of a foreign protein.

This subject may be summarized as follows:

1. Passive sensitization by placental transmission of anaphylactic antibody may occur among the lower animals and possibly among human beings; this sensitization is of short duration.

2. Natural allergy by prenatal active sensitization does not occur at all, or, at least, is apparently a rare and unusual occurrence.

3. The tendency to acquire sensitization is transmissible and especially if both parents are allergic to some substance or substances.

4. Parents do not transmit their own hypersensitiveness, but a tendency to acquire hypersensitiveness to the same or different substances.

5. The nature of the inherited tendency for acquiring hypersensitiveness is unknown.

6. When the tendency for acquiring hypersensitiveness has been inherited, the individual may become allergic to many different substances during the course of a lifetime.

GENERAL LESIONS AND SYMPTOMS OF HUMAN ALLERGY

The lesions of allergy in man are largely disturbances of the vasomotor system. Hyperemia with serous exudation and resulting edema are the most frequent, and according to the organ or organs involved, contribute most of the symptoms.

The lesions and symptoms of allergy in man usually occur in the respiratory, alimentary, and cutaneous systems. When the exciting agent is inhaled, as in pollen and dust allergies, or produced by bacteria upon the mucosa of the respiratory tract, the allergic lesions and symptoms are largely confined to these organs. Likewise in alimentary allergy swallowing of the exciting agent generally produces local lesions and symptoms, but may likewise elicit lesions and symptoms in the respiratory and cutaneous systems.

Allergic Bronchial Asthma.—In 1910 Meltzer¹ suggested that asthma may be in some cases an anaphylactic stenosis of the fine bronchi. Since then numerous investigators have shown this to be true. Goodale² soon demonstrated the allergic nature of horse asthma by skin tests induced by the application of horse-serum to abrasions, and these observations were quickly followed by those of Talbot³ showing sensitization to eggs and milk as causes of asthma in children. The very extensive investigations of Walker soon followed and afforded conclusive proof of the allergic nature of some cases of asthma.

Walker⁴ has found that the proteins of bacteria commonly found in the sputum of asthmatics may be the primary exciting agents, and notably *Staphylococcus aureus*. Matthews,⁵ Rackemann,⁶ and others have verified these observations demonstrating sensitization to the various strains of streptococci, staphylococci, pneumococci, and other bacteria by means of skin tests.

Walker⁷ has also shown that asthma may be due to allergy to the proteins of horse hair and dandruff as well as to the hair, and emanations of the

¹ Jour. Amer. Med. Assoc., 1910, 55, 1021.

² Boston Med. and Surg. Jour., 1914, 171, 695.

³ Boston Med. and Surg. Jour., 1914, 171, 708.

⁴ Jour. Med. Res., 1916-17, 35, 487.

⁵ Medical Record, 1913, 84, 507.

⁶ Jour. Immunology, 1920, 5, 373.

⁷ Jour. Med. Res., 1916, 35, 497.

cat, dog, rabbit, mouse, and other of the lower animals, including the feathers of chickens and geese; Ratner¹ and Larsen and Bell² have reported asthmas in children due to the inhalation of rabbit hair. These individuals suffer with hay-fever-like symptoms when in close proximity to the animal to which sensitization exists, and in not a few cases persistent bronchitis and asthma have been found due to the use of pillows stuffed with the feathers or hair of animals to which individuals were allergic. Gerdon³ has recently described instances of asthma among hair dyers which he ascribed to the inhalation of the dyes belonging to certain p-phenyldiamin derivatives.

Walker⁴ has also observed occasional cases of asthma due to allergy to common foods including those of animal, vegetable, and fruit origin. Talbot and Schloss were among the first to describe cases of food allergy among children with asthmatic attacks and particularly in allergy to eggs. Indeed, the allergic asthmas of children are usually due to foods rather than to bacterial proteins.

Probably the asthma due to pollen allergy is best known, and was described by Blakely and other pioneer investigators in hay-fever. This type of asthma is usually present during the spring and autumn hay-fever seasons; not all persons subject to pollen allergy suffer with asthma, this symptom being present only in those who are especially sensitive.

Even drug allergy may be accompanied by asthma; I know of a physician who suffers with eczema and asthma a few hours after handling arshenamin powder.

Aside from these well known causes, cases of asthma are frequently encountered apparently belonging to this group etiologically, but without the exciting agent being discoverable. For example, I know of several cases of asthma in which the attacks are brought on by the inhalation of coal smoke and gas, the exciting agent being either carbon particles or gaseous substances. Others are rendered asthmatic by street and house dust, etc. These cases are generally included in the group of *dust asthmas* and Cooke⁵ has recently shown the presence of a substance in most house dusts bearing an important relation to asthmas of this origin; hay dust was also found capable of acting as an allergen and not solely a simple mechanical irritant.

Bronchial asthma is, therefore, a common symptom of allergy in man. It is a type of dyspnea due to difficult inspiration or expiration, or both, and apparently caused not only by contraction of the smaller bronchi, but to hyperemia and edema of the mucosa as well. Of course not all asthmas are of allergic origin; asthma of renal, cardiac, and thymic causes, as well as to enlargement of the bronchial glands, are to be carefully differentiated. The causes of allergic asthma, however, are so many and diverse that this type of asthma should always be kept in mind; these may be divided into four groups as shown on page 653.

¹ Amer. Jour. Dis. Child., 1922, 24, 346.

² Amer. Jour. Dis. Child., 1922, 24, 441.

³ Ztschr. f. Gewerbehygiene, 1920, 8, 201.

⁴ Jour. Med. Research, 1916, 35, 509; *ibid.*, 1917, 36, 231.

⁵ Jour. Immunology, 1922, 7, 147.

I. <i>Inspiratory Type</i> due to:	(a) Pollens—Hay-fever.	<ol style="list-style-type: none"> 1. Dandruff and hair of horse, cat, dog, etc. 2. Feathers. 3. Flour dust. 4. Orris root. 5. Face powders and especially rice powder. 6. House and hay dusts.
	(b) Animal emanations and dusts.	
	(c) Drugs, as arsphenamin dust.	
II. <i>Alimentary Type</i> due to foods:	(a) Various meats.	
	(b) Fish and shell-fish.	
	(c) Milk and eggs.	
	(d) Grains, as oats, wheat, rye, etc.	
	(e) Vegetables, as peas, beans, potato, etc.	
	(f) Fruits, as orange, banana, etc.	
III. <i>Bacterial Type</i> due to:	(g) Nuts, as walnut, pecan, etc.	
	(h) Drugs.	
	(a) Bacteria in the upper and lower respiratory tracts.	
IV. <i>Parenteral Type</i> due to:	(b) Foci of bacteria and mucoid secretions in the nose and accessory sinuses, etc.	
	(a) Subcutaneous and intravenous injections of horse-, sheep-, or other alien serum or blood.	
	(b) Injections of drugs.	

Allergic Rhinitis.—Closely allied to allergic bronchitis and asthma is *perennial vasomotor rhinitis*, in which the physical findings in the nose are quite similar to those during acute hay-fever. The condition occurs from day to day and week to week regardless of the environment or occupation of the patient. The symptoms are largely profuse watery discharge from the nose with respiratory obstruction due to hyperemia and edema of the mucosa. Walker¹ has recently described 20 cases due to allergy to emanations from horses, 6 cases due to cats, and a number to feathers. He speaks of similar symptoms among laboratory workers due to rabbit and guinea-pig hair, likewise of symptoms in bakers and housewives due to cereal flours. I have seen 2 cases in women due to allergy to rice powder employed as a cosmetic.

Rackemann² has recently reported that 45, or 22.7 per cent., of a group of 198 patients with this ailment yielded positive skin tests to various substances including 13 sensitive to orris powder commonly employed in dentifrices, 5 to feathers, 5 to one or more of the cereals, etc.

Analogous to allergic rhinitis are cases of *vasomotor conjunctivitis*, of which *vernal conjunctivitis* is a type. The symptoms are mainly hyperemia with profuse lacrimation in which many eosinophil cells are present. The presence of the latter are always suggestive of an anaphylactic reaction. Vernal conjunctivitis is particularly likely to occur during the spring months of the year and may be an allergic lesion.

Allergic Skin Lesions (Urticaria, Angioneurotic Edema, Eczema, Erythema Multiforme).—Allergy is one of several causes of *urticaria* and *angioneurotic edema*. Probably the urticaria of serum allergy (serum sickness) is best known in this connection and has been thoroughly studied and described by von Pirquet and Schick and others. A common mistake, however, is to place too much importance upon allergy as a cause of chronic urticaria. Schloss³ has recently reported that only 10 cases of a series of 50 yielded positive skin reactions to food proteins, and 6 of these reacted to

¹ Jour. Amer. Med. Assoc., 1920, 75, 782.

² Amer. Jour. Med. Sci., 1922, clxiii, 87.

³ Amer. Jour. Dis. Child., 1920, 19, 445.

many proteins of the common foods; tests were not made with bacterial or pollen proteins or at different intervals. According to McBride and Schorer,¹ allergies to fish, tomatoes, and cheese are especially likely to show urticaria, while the cereals and pork produce erythemata in a considerable proportion of cases.

Cases of *angioneurotic edema* (Quincke's disease) are sometimes due to allergy and particularly to foods. Schloss found 3 cases in a series of 11 yielding positive skin reactions, one to milk and wheat and the other to egg and beef. The causal relationship was demonstrated in both by dietetic experiment. Both patients suffered from recurring attacks of local edema of skin recurring every few weeks and lasting for a day or two. Highman and Michael² have studied 14 cases of chronic urticaria and angioneurotic edema, and in a thorough and splendid paper have expressed their belief in allergy to different proteins, and especially foods, as one cause for these conditions. Phillip³ has recently described cases occurring in young dogs caused by food allergies.

Next to urticaria probably *eczema*, and especially *eczema* of children, has attracted most attention as an allergic lesion. The relationship of *eczema* to allergy has been largely studied by means of skin tests, and a common error has been to conclude that the *eczema* was allergic when positive reactions were observed with the allergens of one or more foods. There can be no doubt that some cases are of an allergic nature, but not in as high proportion as commonly believed.

In 1916 Strickler and Goldberg⁴ found positive skin reactions in a small group of cases to various food allergens. White⁵ reported 66 per cent. of positive reactions in a group of cases, and Talbot⁶ observed fourteen positive reactions to egg-white and other food proteins in a series of 16 cases of *eczema* in infants and children. Schloss⁷ observed that 77.4 per cent. of a series of cases in children under sixteen months of age yielded positive skin reactions to various food allergens; among children over sixteen months of age 41.6 per cent. yielded positive reactions. O'Keefe⁸ applied the skin tests in 70 cases of infantile *eczema*, and found that 29, or 41 per cent., gave a positive reaction to one or more foods, egg-white being most commonly observed. Blackfan⁹ has called attention to the fact that many asthmatic children suffer from *eczema* in infancy or early childhood. Of 43 patients without *eczema*, only one showed susceptibility to protein by skin tests, while of 27 patients with *eczema*, 22 yielded positive reactions, and particularly to cow's milk and woman's milk. Usually positive reactions were observed to several proteins. Ramirez¹⁰ has recently reported that 38 of a series of 78 cases of *eczema* yielded positive skin reactions to one or more proteins.

These investigations leave no doubt but that some cases of *eczema* are due to food allergy, but as pointed out by Fox and Fisher,¹¹ the significance of positive skin reactions in these cases is by no means clear; children may react in a positive manner to food allergens which produce no evidences of ali-

¹ Jour. Cutan. Dis., 1916, 34, 55.

² Archiv. Dermat. and Syph., 1920, 2, 544.

³ Jour. Amer. Med. Assoc., 1916, 66, 249.

⁴ Jour. Amer. Med. Assoc., 1922, 78, 497.

⁵ Boston Med. and Surg. Jour., 1918, 175, 5.

⁶ Med. Clinics North America, 1918, 985.

⁷ Amer. Dis. Child., 1920, 19, 433.

⁸ Boston Med. and Surg. Jour., 1920, 183, 569.

⁹ Amer. Jour. Med. Sci., 1920, clx, 341.

¹⁰ Archiv. Dermat. and Syph., 1920, 2, 365.

¹¹ Jour. Amer. Med. Assoc., 1920, 75, 907.

mentary disturbance. Not infrequently and, indeed, usually, reactions occur to more than one food, and withdrawal of these articles from the diet is not always followed by improvement. Up to the present time allergic eczemas have been identified almost solely with food substances; in children doubtless these are of most importance, but in adults bacterial and other allergens are to be kept in mind as bearing a possible relationship to the lesions and symptoms. Eczema in adults is sometimes caused by allergy to a drug; I know of a physician who suffers with eczema of the hands after preparing solutions of arsphenamin and neo-arsphenamin.

Various *erythemas* may be caused by allergies to foods; sometimes serum allergy is accompanied by scarlatiniform rashes. Hazen¹ has described a case of severe *erythema multiforme* due to allergy to the oyster.

Skin rashes are especially common in drug allergies, and may be macular, papular, vesicular, urticarial, bullous, or hemorrhagic.

Allergic Vomiting, Diarrhea, and Abdominal Pain.—In allergies to foods the ingestion of the allergen may produce almost immediately swelling of the lips and tongue and presumably of the stomach and intestines unless vomiting occurs. In less sensitive individuals symptoms of nausea, vomiting, flatulence, abdominal pain, and diarrhea may develop within a few hours, due, presumably, to hyperemia and edema of the gastro-intestinal mucosa. Richet and Girons² have drawn particular attention to these cases of alimentary anaphylaxis, and Duke³ has recently described a series of cases in which abdominal pain was apparently caused by intestinal allergy.

Allergy to Serum (Serum Sickness).—With the advent to antitoxin treatment of diphtheria in 1894 the injection of immune horse-serum quickly became a common practice in the treatment of disease; almost immediately cases of untoward effects were observed and reported, although occasional cases were previously recorded following transfusion with lamb's blood. Among the earliest cases of serum disease following the injection of diphtheria antitoxin was the immediate and fatal attack of a son of Professor Langerhans. This case created a profound impression upon the medical profession and at the present time many physicians, fearing anaphylaxis, hesitate to administer diphtheria antitoxin and other sera for prophylactic and therapeutic purposes, these fears being shared by many of the laity.

The Nature of Serum Disease or Serum Sickness.—Inasmuch as the reactions followed the injection of horse-serum, the condition became known as "serum disease."

This name was applied by von Pirquet and Schick⁴ to the *various clinical manifestations, such as eruptions, fever, edema, and pain in the joints, following the injection of horse-serum*. These symptoms are due to the horse-serum itself, for, as was early shown by Johannessen,⁵ Bokay,⁶ and others, they may manifest themselves after the injection of sterile normal horse-serum. The serum, moreover, of certain horses appears to be more likely than that of others to cause these symptoms, thus accounting for the fact that one lot of antitoxin will cause a higher percentage of serum sickness than will another. A concentrated serum is not so likely to produce serum sickness as whole serum, owing partly to the fact that smaller doses of it are given. According to Rolleston and Ker, the frequency of serum sickness is, as a

¹ Jour. Amer. Med. Assoc., 1914, lxii, 695.

² Bull. d. l'Acad. d. Med., 1920, 34, 625.

³ Arch. Int. Med., 1921, 28, 151.

⁴ Die Serumkrankheit, Leipzig, Deuticke, 1905.

⁵ Deutsch. med. Wchn., 1895, 21, 855.

⁶ Jahresb. f. Kinderh., 1897, xlv, 133.

rule, in direct proportion to the amount of serum given, and in inverse ratio to the severity of the attack; in other words, we may expect to encounter it most often in mild and moderately severe cases that have received very liberal dosages of serum.

Serum sickness is regarded as a true anaphylactic phenomenon. We are prone to call the severe, fatal, and rare instances of death following serum injection examples of anaphylaxis, and to regard serum sickness as a different condition. Both are fundamentally the same, except that in the first instance the body cells are for some unknown reason unduly and highly anaphylactic. *Fortunately, this undue hypersensitiveness is frequently foreshadowed by the asthmatic or hay-fever-like attacks which the susceptible person may exhibit when he enters stables or is otherwise around horses. It goes without saying that horse-serum should never be given to such persons.*

While serum sickness is usually due to horse-serum for the reason that the horse is so commonly employed in the preparation of various curative serums, the serum of the ox, rabbit, and other animals may induce the same train of symptoms in addition, in some instances, to producing a direct toxic effect.

Types of Sero-anaphylactic Reactions.—An immediate reaction rarely follows the first injection of serum unless the patient is one of those unfortunate but rare persons who in some manner have been rendered highly sensitive to horse protein. In the majority of instances symptoms do not develop for from eight to twelve days, during which time the antibody is being produced; this is the usual *delayed reaction*. When antibody formation has reached a certain point it reacts upon any of the horse-serum that may persist in the cells or circulation, producing the anaphylactic reaction. If the dose of serum has been small, antibody formation goes on as usual, but the serum may not persist in the circulation. Hence symptoms do not develop in such a person, although if reinjected subsequently symptoms will appear. This is one reason why a concentrated serum is not so likely to produce serum sickness, since a smaller quantity of it is injected.

If, however, the patient has received an injection of serum some months previously, a reinjection is likely to be followed by an *immediate* reaction, that is, the symptoms appear within from a few minutes to twenty-four to forty-eight hours. If the first injection had been given a year or more previously, no antibody may be present in the blood, so that an immediate reaction does not occur. If, however, the cells once stimulated are "keyed up" indefinitely, and, accordingly, antibody formation is quite rapid, so that we find symptoms developing in from four to seven days after injection—the *accelerated reaction*. Or a small amount of antibody may be present which gives a mild reaction around the site of serum injection, followed in from four to seven days by a general reaction, this being an *immediate followed by the accelerated reaction*.

As was previously stated, anaphylaxis is specific—that is, a person receiving ox-serum in the first injection would not be affected subsequently by an injection of horse-serum, but only by ox-serum. For this reason it has been recommended that diphtheria antitoxin to be used for prophylactic purposes should be prepared by immunizing cattle, reserving the horse-serum for treatment if the disease should be contracted subsequently.

Relation of Route of Administration of Serum to Anaphylactic Reactions.—The route by which serum is administered to man bears a very important relation to both the severity and time of appearance of the symptoms of anaphylaxis.

Acute anaphylactic shock of the lower animals is produced by the *sudden* introduction of serum into sensitized animals by intravenous injections. The same is true of human beings. The intravenous and subarachnoid injections of serum produce anaphylactic effects more quickly than intramuscular and subcutaneous injections, while interal administration by mouth or colonic injection are least likely of all to produce these effects.

With those exceptional human beings who are very highly sensitized to horse protein as the "horse asthmatics," even the subcutaneous injection of serum may result in an immediate reaction developing within a few minutes, but with the great majority of persons the subcutaneous or intramuscular injection of horse-serum is followed by a period of several days to two weeks or more before the onset of symptoms.

Relation of Amount of Serum Administered to Anaphylactic Reactions.—

Ordinarily the administration of purified or concentrated serum does not produce as high an incidence of serum sickness as whole serum. Horse-serum contains at least three sensitinogens: euglobulin, pseudoglobulin, and albumin. Concentrated antitoxin sera prepared by the Gibson-Banzhof method is composed largely of the pseudoglobulins which carry most of the antitoxin; the employment of these concentrated sera means that there is a reduction of the total protein in relation to the antitoxic value and the elimination of all or most of the euglobulins and albumins lessens very materially the chances of inducing serum sickness and the production of recurrent eruptions.

Even with whole serum, smaller amounts, injected subcutaneously or intramuscularly, are less likely to produce serum sickness than larger amounts. With intravenous injections there is less relation and among "horse asthmatics" a few centimeters by any route may induce as much reaction as larger amounts.

Danger of Anaphylactic Reactions.—The occurrence of immediate reactions and fatalities after the administration of horse immune sera employed for prophylactic and curative purposes, has unduly impressed many physicians and engendered a fear of producing reactions which is shared by many of the laity; one result has been hesitation in the administration of diphtheria and tetanus antitoxins for the prophylaxis and treatment of these infections. For this reason a general and brief statement on the dangers of anaphylaxis in man may be in order:

1. Bearing in mind that thousands upon thousands of injections of horse immune sera and especially diphtheria and tetanus antitoxins are administered throughout the world each year, the incidence of *fatal* anaphylactic reactions is very small (probably about 1 in 50,000 to 100,000 injections). The exact incidence cannot be stated because not all cases are recorded, but several investigators have reported the absence of fatal reactions in series as large as 200,000 injections. Furthermore, some of the fatalities attributed to the anaphylactic effects of the serum may have been caused by other factors. Approximately 50 fatalities after the administration of serum have been recorded.

The incidence of severe reactions during or immediately after the intravenous or subarachnoid injections of serum is much larger, but recovery is the rule. Likewise the incidence of mild anaphylaxis designated as "serum sickness" or "serum disease" is high, but this reaction is without danger.

2. Individuals, including children as well as adults, who are uncomfortable around horses and are seized with sneezing, coughing, "asthma" and other hay-fever-like symptoms, are extremely sensitive to horse protein and should not receive injections of horse-serum by any route. Apparently

some of the small group of fatalities following the administration of serum have occurred among these persons. A physician called upon to administer serum should always first inquire upon this point; if the history is suggestive a skin test may be conducted, as described in a succeeding chapter. Desensitization may likewise be tried as described later, but in a typical "horse asthmatic" the administration of horse-serum is dangerous despite these precautions.

Children with status-thymo-lymphaticus are likewise bad risks; this condition is regarded by some investigators as responsible for some of the fatalities following the administration of serum.

3. Individuals who have received injections of serum upon a previous occasion are almost sure to develop "serum sickness" after reinjection, but this is not dangerous, although distressing, and should not be a contradiction to the use of serum. Serum may be administered by subcutaneous or intramuscular injection with safety.

When serum is given intravenously to such individuals, preliminary desensitization should be practised. This is likewise advisable before the intraspinal injection of serum. Methods are given in a subsequent chapter.

Symptoms of Immediate and Severe Anaphylactic Shock.—These are characterized by sudden onset—often within a few minutes after or during the intravenous injection of serum. The patient becomes restless, anxious, and may give an outcry; there is marked pallor, perspiration, rapid and feeble pulse. Respirations become deep, labored and sometimes rapid, and the patient may become unconscious. Muscular twitchings, rigors, and convulsions may occur with micturition and defecation. In rare instances death occurs during coma with respiratory failure, while the heart is still beating.

Some of these symptoms may develop during the intravenous injection of serum, but subside as soon as the injection is stopped. Death does not occur except in those rare cases of extreme sensitizations to horse protein; in these even the subcutaneous injection of serum may bring on the reaction with a fatal outcome.

Not infrequently a reaction of fever, chills, rapid pulse, and prostration develop an hour or two after the intravenous injection of serum; this reaction is probably not anaphylactic, but protein shock.

Symptoms of the Usual Reaction (Serum Disease).—*The most typical of these symptoms are rash, fever, and prostration, besides joint and muscle pains, edema, and adenitis.*

These symptoms and lesions may develop within a few hours to two weeks or so after the intravenous, intraspinal, intramuscular, or subcutaneous injection of serum. In the majority of cases the reaction develops eight to twelve days after the injection, although it may appear within the first few days and especially if the individual had received an injection of serum on a previous occasion.

Frequency.—The frequency of these reactions varies greatly in the different recorded series of cases. It would appear that the incidence in Germany (8.1 per cent.) and France is much lower than in America. The incidence in this country has varied with the sera of different horses and has been generally lower with concentrated (purified) sera than with whole sera.

Reactions are slightly more frequent after intrathecal injections of serum than after subcutaneous injections. The following table gives the approximate incidence from different parts of the world:



FIG. 152.—URTICARIAL RASH OF SERUM SICKNESS.

Case of laryngeal diphtheria; had received 40,000 units of antitoxin eight days previously; urticarial rash first appeared about thirty hours before this drawing was made.

INCIDENCE OF SERUM DISEASE

REPORTED BY	ROUTE OF ADMINISTRATION.	SERUM.	INCIDENCE, PER CENT.
Flexner	Intrathecal	Whole	70
Rolleston	"	"	41 to 60
Drought	"	"	55
Goodall	Subcutaneous	"	35 to 40
Hartung	"	"	11.4
Rolleston	"	"	44
Dant	"	"	11.4
Coca	"	Purified	8.5
Kolmer	"	"	10

In a general way it may be stated that the administration of *whole horse-serum* by subcutaneous, intramuscular, intravenous, and intrathecal injection is followed by serum disease in 40 to 60 per cent. of cases; the subcutaneous or intramuscular injection of *purified horse antitoxin serum* is followed by serum disease in approximately 10 per cent. of cases.

Incubation Period.—Symptoms may appear in fifteen minutes after the intravenous injection of whole serum. In about 10 per cent. of cases developing serum sickness the symptoms appear within the first four days; the majority (about 60 per cent.) of cases develop symptoms in from six to ten days after injection and these are the critical days.

Fever.—After the intravenous injection of serum a thermal reaction may develop within one hour or two, but this is usually due to non-specific protein shock rather than to anaphylaxis. In serum disease the temperature usually rises one or two days before the rash or on the same day just as the rash appears; it is well for the physician to keep this in mind in order to guard against concluding that this fever is due to reinfection or exacerbation of infection. Sometimes fever is absent and especially in mild cases.

Eruption.—The most obvious and important of the symptoms are undoubtedly the various forms of *rash*. In 1000 consecutive cases of diphtheria treated with antitoxin in the Philadelphia Hospital for Contagious Disease, a rash developed in 430, or 43 per cent. The time of appearance of the eruption depends, as was just stated, upon whether or not the patient has been injected on a previous occasion, and if so, the length of the interval between the first and subsequent injection, and to a lesser extent upon the amount of the first injection, these factors influencing the quantity of antibody present at the time of reinjection. Of the 430 cases just mentioned, the time of appearance of the rash, in days, after subcutaneous injection of antitoxin was as shown in table on page 660.

In that table are included some cases of reinjection, as, *e. g.*, scarlet fever patients who received a routine immunizing dose of antitoxin upon admission, and another after having contracted diphtheria; also cases of diphtheria that became reinfectd within a few months after their discharge from the hospital. As will be seen, about 63 per cent. of cases develop a rash between the sixth and the ninth day after the injection of antitoxin.

Three main types of rashes are generally recognized:

1. *Urticarial Rashes.*—If we include in this group all eruptions that present a resemblance to urticaria, these rashes are the most common, constituting from 70 to 90 per cent. of all eruptions. They usually appear after the seventh day, becoming manifest first about the site of injection. Large, irregularly shaped, and scattered blotches appear, frequently with true wheals in the center (Fig. 152), accompanied by intense itching and

TIME OF APPEARANCE OF SERUM RASH IN 430 CASES OF SERUM DISEASE

DAY UPON WHICH THE RASH APPEARED AFTER INJECTION OF DIPHTHERIA ANTITOXIN.	TOTAL NUMBER SHOWING RASH.	PERCENTAGE.
First.....	4	0.9
Second.....	6	1.4
Third.....	7	1.6
Fourth.....	30	6.9
Fifth.....	35	8.1
Sixth.....	75	17.6
Seventh.....	65	15.1
Eighth.....	85	19.8
Ninth.....	44	10.2
Tenth.....	39	9.0
Eleventh.....	22	5.1
Twelfth.....	5	1.1
Thirteenth.....	6	1.4
Fourteenth.....	5	1.1
Fifteenth.....	1	0.2
Sixteenth.....	1	0.2

irritation. Sometimes true wheals do not appear. The rash is often very profuse, and fresh blotches may continue to appear for two or three days. Occasionally, the rash is quite sparse and mild, and may disappear within twenty-four hours.

2. *Multiform Rashes*.—This type of rash is quite common. It is often circinate in its arrangement, or occurs in large blotches mixed with a scattered morbilliform or measly form of rash (Fig. 153). Different parts of the body may present different appearances at the same time. This rash may occasionally closely simulate true measles, especially since it involves the face, and as the conjunctivæ are likely to be congested in almost any variety of serum sickness. It is differentiated from measles by the fact that Koplik's spots are absent, there is no prodromal rise in temperature, the papules are not elevated above the skin as much as in measles, and that the eruption frequently starts from the site of injection, instead of on the face.

3. *Scarlatiniform Rashes*.—This type of rash occasionally occurs, and may bear so close a resemblance to true scarlet fever as to be indistinguishable from it. The eruption usually appears early, that is, in from the first to the sixth day after injection, and may vary from a uniform erythema which *first appears about the site of injection*, to a true punctate scarlatiniform rash. The differentiation of these rashes from the true scarlet fever eruption is one of the greatest sources of trouble in hospital practice, especially when they develop in the diphtheria wards, where occasional cases of true scarlet fever are always likely to appear from time to time. The absence of the following symptoms, or their occurrence only in mild degree, would favor a diagnosis of serum disease: Fever, or, at least, the presence of but a mild pyrexia, the vomiting, the typically furred tongue, the angina, or at least but a mild throat involvement, and the leukocytic inclusion bodies—all forming the symptom-complex of true scarlatina. In many instances, however, it is necessary to isolate the patient, when speedy recovery, absence of complications, and less definite desquamation, which does not involve the palms and soles, indicate that the patient was suffering from serum disease and not from scarlet fever.

As previously stated, *the eruption is at first usually local about the site of injection of serum* and several days may elapse before it becomes generalized. In some mild cases the eruption remains localized. Recurrent eruptions



FIG. 153.—MULTIFORM RASH OF SERUM SICKNESS.
Child with laryngeal diphtheria on the sixth day after receiving 65,000 units of antitoxin.

sometimes occur. Occasionally there may be considerable edema present with the skin rash, or it may occur independently. It is most common about the face and neck and may be so extensive as to close the eyes; this usually lasts only a few hours.

Arthritis; Neuritis; Myalgia.—Painful and tender joints occur in about 2 per cent. of cases. The process seems to be extra-articular; there is seldom any fluid in the joints, nor is the skin hot or reddened. There may be slight periarticular edema. Boots and Swift,¹ however, have recently stated that fluid was found in the joints of four of six cases studied and that there were cytological changes of acute arthritis. Furthermore, horse-serum was found in two fluids, suggesting "that the irritation of the joint may be due to the presence of this foreign protein in an allergic tissue." All the joints may be attacked or only one or two; the temporomandibular and metacarpophalangeal joints appear to be more frequently affected than other joints. Neuritis, myalgia, and headache may also occur.

Adenitis.—The regional lymph glands and especially those nearest the site of subcutaneous injection of serum frequently become swollen and tender. These lesions are among the first to appear and first to disappear. Generalized adenitis may develop. Suppuration does not occur. Occasionally the cervical glands beneath the sternomastoid muscle may become so enlarged and tender as to suggest a pyogenic infection, but this usually disappears in two to three days. Occasionally the spleen becomes palpable.

Leukocyte Changes.—In the first few hours of serum disease there is likely to be a slight leukocytosis with an increase in the relative number of lymphocytes (30 to 40 per cent); this is followed by leukopenia. Frequently there is a slight increase of the eosinophil cells (3 to 7 per cent.).

General Symptoms.—Tachycardia may occur even when the temperature is approximately normal. Diarrhea may develop and prove a troublesome symptom for several days. There is rarely nausea and vomiting. Marked prostration of the neuromuscular system may occur followed by an asthenia of many days or weeks.

Mild nephritis with slight albuminuria is sometimes present. Longcope found about 10 per cent. of cases showed albuminuria and casts. There is little change in the coefficient of urea excretion or in the phthalein output. The excretion of water and chlorids are rapidly diminished at the onset, but these renal changes are transitory and the return to normal is usually rapid.

Delahet² has recently described meningeal symptoms of anaphylactic nature following repeated intrathecal injections of serum.

Recurrent Serum Disease.—Mention has already been made of the "double reactions" of von Pirquet and Schick, the "accelerated" following the "immediate" reaction. A number of observers have reported second eruptions following in three to twenty-one days after the first eruption. Goodall³ has described 2 cases of triple eruptions; in the first case the eruptions appeared on the first, third, and seventh days, and in the second case they occurred on the second, seventh, and tenth days following reinjection. Axenow⁴ reports that of 683 cases of serum disease, 10 per cent. developed a triple eruption and 2.3 per cent. a quadruple eruption.

These recurrent eruptions appear to be caused by more than one sensitizing in the serum; as reported by Coca they do not occur with the use of purified (concentrated) antitoxin sera.

¹ Jour. Amer. Med. Assoc., 1923, 80, 12.

² Bull. d. l. Soc. Med. d. Hôp., 1920, 44, 1272.

³ Jour. Hyg., 1907, 7, 607.

⁴ Jahrb. f. Kinderh., 1913, lxxviii, 565.

The Detection, Prevention, and Treatment of Serum Disease.—Anaphylactic phenomena can usually be expected to occur if serum is given within a year or so following a previous injection. Persons who experience discomfort when about horses should always be closely questioned. By means of skin tests the state of allergy to serum may be detected and especially in those cases of extreme hypersensitiveness to horse protein. These preliminary tests are particularly indicated before the intravenous and intrathecal injection of serum when the patient is known to have had an injection of serum upon a former occasion. When serum is to be given subcutaneously or intramuscularly they are not necessary unless the physician suspects that the patient is hypersensitive to horse protein. The technic and a fuller discussion are given in the succeeding chapter.

Sero-anaphylactic reactions may be prevented or at least greatly reduced in severity by preliminary desensitization before serum is administered. This is particularly indicated before the intravenous and intrathecal injection of serum. Methods are described in Chapter XXXII.

For the immediate reaction sometimes developing within fifteen minutes after the administration of serum and especially by intravenous and intrathecal injection, *adrenalin chlorid* (1 : 1000) should be injected intramuscularly in dose of 1 c.c. (℥xvj). *Atropin sulphate* should also be injected subcutaneously in dose of 0.5 mg. (1/120 grain). *Whenever serum is given intravenously it is a good routine practice to have these two medicaments ready in syringes for immediate use;* usually they are not required, but if needed, no time is lost in the administrations. If the pulse is particularly soft and rapid stropanthin may be given intramuscularly in dose of 0.5 mg. (1/120 grain) or an equivalent preparation of digitalis.

In the usual case of serum sickness, the urticarial rash is the most distressing symptom. A brisk cathartic should be given. The subcutaneous injection of adrenalin chlorid 1 : 1000 in dose of $\frac{1}{2}$ to 1 c.c. (℥vij to xvj) often affords instant relief which may persist for several hours. I usually give an injection late in the evening in order that the patient may have a few hours of sleep. Calcium chlorid and lactate, in doses of from 3 to 5 grains, have been advocated as a prophylactic and cure, but they are of doubtful utility. Sometimes the administration of a sedative dose of morphin sulphate is indicated along with an injection of adrenalin. A soothing lotion should be applied to the skin, the following well-known calamin lotion being efficacious:

Phenol.....	2 c.c.
Calamin.....	4 gm.
Zinc oxid.....	8 gm.
Glycerol.....	12 c.c.
Lime-water.....	16 c.c.
Water up to.....	120 c.c.—M.

Aspirin may be administered for headache, myalgia, and neuritis. Aspirin or sodium salicylate combined with sodium bicarbonate may be given every four to six hours for the joint pains.

ALLERGY TO POLLENS (HAY-FEVER)

The hypersensitiveness displayed by some individuals to the pollens of various plants is one of the earliest and best known examples of human allergy.

Historic.—In an excellent review of the early literature upon this subject by Hitchens and Brown,¹ mention is made of clinical observations by Botallus as early as 1565, who wrote: "For there are many who are attacked

¹ Jour. Lab. and Clin. Med., 1915-16, 1, 457.

with sneezing, by the slightest thing whatsoever, others by merely smelling a rose." These authors have stated that there is evidence that hay-fever has existed for centuries before its recognition as a specific disease, and all attempts to estimate its antiquity are futile.

In 1819 Bostock¹ contributed a very complete clinical description of hay-fever and expressed the belief that it was caused by the heat of summer. Excellent statistical studies were made by Elliotson² and Phoebus,³ and of special interest in relation to the hereditary tendency for acquiring the disease was the opinion expressed by Perrie⁴ in 1867, that it was of nervous origin.

The most notable contributions were made by Blackley⁵ in 1873. Himself a sufferer with hay-fever, he conducted a remarkable series of experiments constituting one of the most complete researches in the history of experimental medicine. Blackley tested on himself the pollens of the grasses and plants belonging to thirty-five orders, and was first to employ skin and mucous membrane tests for sensitization. He also showed that pollen may be carried for long distances in the air.

The next notable contributions on the etiology of hay-fever were made by Dunbar,⁶ who confirmed the observations of Blackley and expressed the belief that the condition was caused by a pollen toxin. Since then a very large literature has accumulated upon treatment with Dunbar's supposedly antitoxin serum designated as "Pollantin," and more especially with extracts of pollens, as first employed by Curtis⁷ and Noon.⁸

The Nature of Hay-fever.—Hay-fever is now commonly regarded as a condition of hypersensitiveness or allergy to pollens. Some of the earlier investigators considered the disease as a bacterial infection and endeavored to explain the relation of pollens to the production of attacks by assuming that they acted as carriers of bacteria. As a matter of fact, it is now well known that perennial hay-fever-like attacks or vasomotor rhinitis may be due solely to bacterial sensitization and that some cases of hay-fever are apparently caused by sensitizations to both pollens and bacteria.

The older theory of Dunbar that the disease was caused by a pollen toxin analogous to bacterial toxins has been thoroughly disproved. The theory of the allergic nature of pollen disease first proposed by Wolff-Eisner⁹ is now generally accepted, although his explanation of the production of the symptoms and lesions by an "anaphylatoxin" from the digestion of the pollen protein by specific antibody and complement, is not acceptable.

There can be no doubt that the pollens contain protein substances according to the analyses reported by Kammann¹⁰ and Koessler¹¹; Heyl¹² has recently obtained an albumin, a proteose, and a glutelin from the pollen of ragweed. However, it appears that the sensitogens in pollens are but feebly antigenic. Ulrich¹³ and Koessler¹⁴ have reported successful sensitization of guinea-

¹ *Medico-Chi. Trans.*, 1819, 10, 161; *ibid.*, 1828, 14, 437.

² *Lancet*, 1830-31, 2, 370.

³ Gressen, Ricker, 1862, 136.

⁴ *Med. Times and Gaz.*, 1867, 2, 2, 30.

⁵ See Blackley: *Hay-fever, Its Causes, Treatment and Effective Prevention*, 2d ed., London, 1880.

⁶ *Berl. klin. Wchn.*, 1905, Nos. 26, 28, 30.

⁷ *New York Med. News*, 1900, 37, 16.

⁸ *Lancet*, 1911, 1, 1572.

⁹ Wolff-Eisner: *Das Heufieber*, München., 1906.

¹⁰ *Biochem. Ztschr.*, 1912, 46, 151.

¹¹ *Jour. Amer. Chem. Soc.*, 1917, 39, 1470; 1919, 41, 670.

¹² *Jour. Amer. Chem. Soc.*, 1919, 41, 670.

¹³ *Jour. Immunology*, 1918, 3, 455.

¹⁴ *Forchheimer's Therapeutics of Internal Diseases*, 1914, 5, 686.

pigs with pollens, but Cooke, Flood and Coca,¹ and Smith² were unable to sensitize guinea-pigs with them. Koessler also claims to have successfully sensitized guinea-pigs in a passive manner with the sera of sensitized human beings. Clowes³ observed that subcutaneous injection into a hay-fever subject of a pollen protein to which the individual is not naturally hypersensitive does not induce clinical hypersensitiveness to that protein.

Furthermore, only a small percentage of human beings acquire hay-fever (10 per cent. in the United States, according to Cooke and Vander Veer). This indicates that some predisposing factor must be present rendering the body cells of some individuals more susceptible of sensitization with pollens than is the case of most persons and the lower animals.

This predisposition to sensitization is apparently inherited. Some of the earliest writers on hay-fever observed family predisposition, and finally Cooke and Vander Veer were recently able to show quite conclusively the hereditary transmission of the *tendency* to become hypersensitive to various protein substances including the pollens. Actual sensitization is not inherited, but the predisposition to become sensitized is, the subject having been discussed at more length in a preceding chapter.

Pollens Causing Hay-fever.—These are broadly divisible into two main groups: (a) Those causing early or spring fever, running from the middle of May to the first or middle of July, and (b) those causing late or autumnal fever, the attacks usually beginning in the middle of August and running to the first frost. Cooke and Vander Veer have given the following list as commonly producing the spring or early hay-fever:

<i>Common Name.</i>	<i>Botanical Name.</i>
Timothy	Phleum pratense
Red-top	Agrostis alba
June grass	Poa pratensis
Sweet vernal	Anthoxanthum odoratum
Low spear	Poa annua
Orchard grass	Dactylis glomerata
Rye	Secale cereale
Wheat	Triticum sativum
Quickgrass	Agropyrum repens
Locust	Robinia pseudo-acacia
Chestnut	Castanea dentata
Maple	Acer rubrum
Daisy	Chrysanthemum leucanthemum
Rose	Rosa
Honeysuckle	Lonicera caprifolium
Privet	Ligustrum vulgare

The following is a list of those pollens usually responsible for the late or autumnal cases:

<i>Common Name.</i>	<i>Botanical Name.</i>
Ragweed	Ambrosia trifida
Ragweed	Ambrosia artemisiifolia
Goldenrod	Solidago canadensis
Goldenrod	Solidago nemoralis
Chrysanthemum	Chrysanthemum
Dahlia	Dahlia
Zenia	Zenia
Clematis	Clematis Virginiana
Marsh grass	Spartina stricta
Aster	Aster

¹ Jour. Immunology, 1917, 2, 217.

² Jour. Immunology, 1918, 3, 325 (disc.).

³ Jour. Immunology, 1918, 3, 325 (disc.).

Cooke and Vander Veer found that with few exceptions individuals sensitive to one of the grasses reacted to all, which indicates a biologic relationship of the proteins derived from the pollens of the graminaceæ. Furthermore, desensitization effected with the pollen of one grass usually reduces hypersensitiveness to all, as will be discussed in more detail in the succeeding chapter.

Likewise individuals who are hypersensitive to ragweed react to the pollens of the two varieties, and the same is true of individuals hypersensitive to goldenrod reacting to both varieties of this plant, indicating that these pollens are biologically identical while botannically different. But individuals who are sensitive to ragweed may not be sensitive to goldenrod, and the reverse.

Symptoms of Hay-fever.—The description of a case by Bostock in 1819 adequately summarizes the usual symptoms and lesions of hay-fever and especially of the late or autumnal type which is apt to be more severe than the spring variety and frequently accompanied with asthma. "A sensation of heat and fulness is experienced in the eyes, first along the edges of the lids, and especially in the inner angles, but after some time over the whole of the ball. At the commencement the external appearance of the eye is little affected, except that there is a slight degree of redness and a discharge of tears. This state gradually increases, until the sensation becomes converted into what may be characterized as a combination of the most acute itching and smarting, accompanied with a feeling of small points striking upon or darting into the ball, at the same time that the eyes become extremely inflamed and discharge very copiously a thick mucous fluid.

"After this state of the eyes has subsisted for a week or ten days, a general fulness is experienced in the head, and particularly about the forehead; to this succeeds irritation of the nose, producing sneezing, which occurs in fits of extreme violence coming on at uncertain intervals. To the sneezings are added a further sensation of tightness of the chest, and a difficulty of breathing, with a general sensation of the fauces and trachea. There is no absolute pain in any part of the chest, but a feeling of want of room to receive the air necessary for respiration, a huskiness of the voice, and an incapacity of speaking aloud for any time without inconvenience. To these local symptoms are at length added a degree of general indisposition, a great degree of languor, an incapacity for muscular exertion, loss of appetite, emaciation, restless nights, often attended with profuse perspirations, the extremities, however, being generally cold. The pulse is permanently quickened, from 80, the average standard, to about 100, and upon any considerable exertion it arises to 120 or more."

Diagnosis and Specific Treatment of Hay-fever.—The history and symptoms are usually so characteristic that diagnosis is a simple matter. Sometimes the patient knows from experience the plant or plants to which hypersensitiveness exists, and especially in the late or autumnal type in which the pollens of ragweed or goldenrod, or both, are usually the causes.

Skin tests, however, are readily applied and have proved quite satisfactory for determining the pollens to which sensitization exists. These are described in a succeeding chapter on page 674.

Specific treatment consists in desensitization by means of injections of pollen extracts. This method of treatment is described in Chapter XXXII.

ALLERGY TO ANIMAL SUBSTANCES

It is now well known that some individuals suffer with asthma, vasomotor rhinitis, and other hay-fever-like symptoms when in contact with certain of the lower animals, notably the horse.

Meltzer¹ suggested in 1910 that this type of asthma may be an anaphylactic phenomenon, and Walker, Rackeman, and other investigators have since shown this to be the case.

Allergies of this kind may be acquired to the hair, dandruff, and expired air of the horse, rabbit, guinea-pig, and other of the lower animals; likewise to goose feathers. In the latter instance sleeping upon goose feather pillows may elicit attacks of vasomotor rhinitis and asthma.

The degree of sensitization may be so extreme that allergic attacks are elicited by proximity to the animal sensitinogen, without actual contact. Not infrequently the exciting agent is carried in dust and constitutes one form of allergenic dust asthma.

The tendency to acquire these sensitizations is sometimes apparently inherited. Injections of horse-serum rarely result in the production of such extreme degrees of hypersensitiveness.

The **diagnosis** of this type of allergy is sometimes quite easy, as the patient frequently knows the animal or animals to be avoided. Skin tests have proved of considerable diagnostic value and are described in the succeeding chapter. Individuals may be hypersensitive to the hair of an animal and not to its serum; as a general rule, however, hypersensitiveness to the horse includes sensitization to hair, dandruff, and serum, and for this reason the administration of horse-serum in the prophylaxis and treatment of disease may be very dangerous for such persons.

To be included in the category of allergies to animal substances are the excessive effects experienced by some individuals to the *stings of insects*. Longcope² has reported one individual in whom the sting of the mosquito produces enormous areas of edema similar to the so-called angioneurotic form, and in another case a sting of a wasp was followed by symptoms exactly like the immediate reaction in serum disease.

ALLERGY TO PLANT SUBSTANCES (DERMATITIS VENENATA)

The extreme hypersensitiveness of some individuals to poison ivy (*Rhus toxicodendron*), poison sumac or dogwood (*Rhus venenata*), poison oak (*Rhus diversiloba*), and the primrose (*Primula obconica*) strongly suggests that allergies to these plants may develop. Recently Simmons and Bolin³ have described a similar condition among some individuals to the mango (*Mangifera indica*).

The exact nature of the exciting agents are unknown; toxicodendric acid and various glucocidal substances are commonly mentioned in this connection. Simpson⁴ has found that protein from primrose did not elicit a reaction, whereas the active principle was found soluble in absolute ethyl alcohol. Simmons and Bolin likewise found that the proteins of the mango did not engender skin reactions, but that a non-protein skin irritant was obtained from the stem sap of either ripe or green mangoes.

Doubtless the juices of these plants contain irritants capable of producing dermatitis by direct irritation and without allergic sensitization. It is common experience, however, that only certain individuals are susceptible and that some of these are so extremely hypersensitive, that attacks of derma-

¹ Jour. Amer. Med. Assoc., 1910, 55, 1021.

³ Amer. Jour. Trop. Med., 1921, 1, 351.

² Amer. Jour. Med. Sci., 1916, 152, 637.

⁴ Jour. Amer. Med. Assoc., 1917, 69, 95.

titis are produced by mere proximity to the plant without actual contact. In these respects dermatitis venenata bears a close analogy to pollen hay-fever and may be an allergic phenomenon, even though the exact nature of the sensitizing agents and the mechanism of sensitization and reaction are unknown. Doubtless a *tendency* to acquire sensitization to these plants may be inherited, as has been found true of other examples of human sensitizations, and especially so, since the skins of many susceptible individuals appear to be sensitive to other substances. Further evidence of the allergic nature of dermatitis to poison ivy is afforded by the observation of Schamberg that the internal administration of the tincture or fluidextract may bring about rapid but temporary amelioration of the symptoms, suggestive of a process of desensitization. As shown by Strickler¹ and Anderson and Pruett² similar results are obtained with the intramuscular injection of the extract.

McNair³ explains the tolerance of most individuals for lobinol (the active principle of *Rhus diversiloba*) on the basis of non-specific factors, and especially the particular structure of the sebaceous and sudoriparous glands and the chemical nature and abundance of their secretions. This tolerance, however, could be overcome by the application to the skin of strong extracts, showing that it was relative and not absolute; similar results were observed by Simmons and Bolin with the sap of the mango. These investigators do not subscribe to the view that dermatitis venenata may be an allergic phenomenon, but that tolerance is dependent upon non-specific factors relating to the skin and that susceptibility is due to a modification or absence of these factors.

Spain⁴ has recently studied a large group of cases of dermatitis venenata and found that the typical vesicular lesion can be produced by applying alcohol and chloroform extracts to the skin, but that these reactions could not be produced by intracutaneous injections. Infants were found to be insusceptible.

ALLERGY TO BACTERIA

Our knowledge of clinical allergy to bacteria is practically confined to a type of asthma occurring with chronic bacterial infections of the upper respiratory tract. These have been the subject of special studies by Walker and Rackemann with the aid of skin tests conducted with the proteins of various bacteria recovered in cultures from the discharges.

Chronic bacterial infections of the accessory sinuses of the upper respiratory tract may be likewise associated with asthma, but this is the only clinical condition clearly identified with bacterial anaphylaxis at the present time. Bacterial allergic asthma is not encountered in children, and is apparently acquired in the later years of life and frequently as a result of previous chronic bronchitis.

It is possible that perennial rhinitis, commonly designated as a vasomotor rhinitis which persists throughout the year, may be an expression of bacterial allergy; likewise some cases of eczema. Skin tests with different bacterial proteins not infrequently yield positive reactions without clinical evidences of the sensitization.

¹ Jour. Amer. Med. Assoc., 1921, 77, 910.

² Californ. State Jour. Med., 1921, 19, 188.

³ Jour. Infect. Dis., 1916, 19, 419.

⁴ Jour. Immunology, 1922, 7, 179.

ALLERGY TO FOODS

The intolerance or idiosyncrasy of certain individuals for certain foods has been known clinically for many years, as attested to by the aphorism: "One man's meat is another man's poison." That is, the immediate symptoms of vomiting, diarrhea, and abdominal pain have been long associated with food idiosyncrasy for the very evident reason that the average adult patient was able to associate cause with effect.

It has been only within the last ten or twelve years, however, that our knowledge of this subject has been greatly extended and principally by the pediatricians and dermatologists, to include one kind of asthma and other untoward effects from foods in children including the development of various skin lesions, notably eczema and urticaria. These studies were given great impetus by the application of skin tests by Smith¹ in the diagnosis of allergy to buckwheat, and Schloss,² Goodale,³ Talbot,⁴ and others in the diagnosis of allergies to raw eggs, milk, and other foods.

Sensitization in Food Allergy.—Among the allergies of children, that for food substances is probably the most common. Possibly active sensitization may be inherited inasmuch as Talbot and others have described instances of allergy to cow's milk in very young infants, the administration of small amounts being followed by immediate vomiting, but this is improbable. Since inherited sensitization is of the passive type and of very short duration in experimental animals, it is more likely that a predisposition to sensitization is inherited and that actual sensitization may result from the passage of foods, eaten by the mother, in her milk. This possibility is shown by the observations and experiments of O'Keefe⁵ and Shannon.⁶ The former found that some nursing infants with eczema gave positive skin reactions to proteins they had never eaten, and the latter observed the withdrawal of certain foods from the diet of a mother to which she was hypersensitive, to be followed by the disappearance of an urticarial eruption on her nursing infant.

Several investigators have shown that young guinea-pigs may be sensitized by feeding. Reference to the experiments of Wells was made in the preceding chapter and Kassowitz⁷ and others have also reported successful sensitizations by feeding. Numerous investigators, including Moro,⁸ Lust,⁹ Hahn,¹⁰ and others, claim to have found precipitins in the sera ascribed to the permeability of the intestinal mucous membrane and the antigenic effects of absorbed foreign proteins.

It would appear, therefore, that sensitization to foods is acquired, although a predisposition to sensitization may be inherited. If active sensitization is not inheritable, then it must be assumed that infants become sensitized by food substances in the mother's milk in order to explain specific sensitization to foods which a child has never eaten. Doubtless inflammatory changes in the gastro-intestinal mucosa aid in sensitization by facilitating the absorption of food sensitinogens. Since proteins digested to the peptone and amino-acid stages are doubtfully allergenic, it is prob-

¹ Arch. Int. Med., 1909, 3, 358.

² Amer. Jour. Dis. Child., 1912, 3, 341.

³ Boston Med. and Surg. Jour., 1916, 175, 181.

⁴ Boston Med. and Surg. Jour., 1914, 171, 708.

⁵ Boston Med. and Surg. Jour., 1920, 182, 569.

⁶ Amer. Jour. Dis. Child., 1921, 22, 223.

⁷ Ztschr. f. Kinderh., 1912-13, 5, 75.

⁸ Münch. med. Wchn., 1906, 49.

⁹ Jahrb. f. Kinderh., 1913, lxxvii, 383.

¹⁰ Jahrb. f. Kinderh., 1913, lxxvii, 405.

able that sensitization can result only from the absorption of undigested or partially digested foods.

Owing to the chemical relationships among certain foods, it is highly probable that sensitization with one may result in sensitization to several. For example, in sensitization to wheat, Goodale has shown that reactions may be produced by oat, barley, and other members of the grass family.

Foods Commonly Producing Allergy.—These include foods of both animal and vegetable origin including some of the fruits, notably the strawberry and gooseberry. Practically every common food has been implicated, and especially buckwheat, pork, oysters, clams, lobsters, cheese, etc. In children the foods mostly involved are cow's milk, eggs, oatmeal, potato, peas, rice, beef juice, and chicken. Raw eggs and uncooked milk have been found especially important in connection with allergic asthma and "exudative diathesis" of children.

Symptoms of Food Allergy.—These may arise from the alimentary, respiratory, and cutaneous systems.

In cases of extreme allergy the alimentary lesions and symptoms are quite marked, and may include swelling of the lips and tongue, nausea, eructations, vomiting, abdominal pain, and diarrhea. In buckwheat allergy I have seen extensive swelling of the tongue, lips, and face occur within a few minutes after the ingestion of a minute amount of buckwheat flour. Infants subject to milk allergy are likely to vomit almost immediately after milk is swallowed.

Asthma may be a symptom and especially in children. Talbot has described cases of croup in children due to allergy to eggs, as likewise recurrent bronchitis. Asthma and vasomotor rhinitis may also occur among adults.

Mention has already been made of edema of the lips and tongue, which may involve the pharynx and larynx in cases of extreme hypersensitiveness. Urticarial rashes resembling serum sickness are of frequent occurrence, as likewise angioneurotic edemas. These lesions may develop within a few minutes to a few hours after ingestion of food. Eczema, erythema multiforme, and other cutaneous lesions may develop as more chronic manifestations of food allergy.

Diagnosis and Treatment.—The diagnosis of acute allergic reactions by foods in adults is usually an easy matter and especially if the symptoms are gastro-intestinal and accompanied by the development of urticaria or angioneurotic edema. The diagnosis of allergic asthma, eczema, and other chronic lesions may require the aid of skin tests, which are described in the succeeding chapter.

Treatment has usually consisted in the total or partial withdrawal of the allergic food or foods from the diet. Desensitization has also been employed and will be described in a succeeding chapter.

ALLERGY TO DRUGS

Drug allergy is a condition of hypersensitiveness in which a quantity of the substance non-toxic for most individuals elicits an unusual but characteristic reaction.

A large number of medicinal substances are known to be active in these allergies, a list being given on page 616. Drug allergies are highly specific; this phase, together with the nature and mechanism of the reaction, are discussed in Chapter XXVIII.

Not all skin eruptions following the administration of drugs can be

designated as allergic; they may be caused by excessive cutaneous elimination and other factors. *Allergy is characterized by the development of symptoms with small and non-toxic amounts borne by most individuals without untoward effects.*

Incubation Period.—In most cases of drug allergy the symptoms appear within a few minutes to a few hours, depending upon the route of administration. In such cases the time of sensitization is usually unknown. In many individuals the symptoms of allergy develop after several administrations covering a period of ten to twenty days, as has been especially observed with arsphenamin.

Drug allergy is sometimes present in very young children and even at birth, but recorded instances of inherited allergy are very few. It is likely, however, that a tendency to acquire drug hypersensitiveness is inheritable, as indicated by the studies of Cooke and Vander Veer in "human sensitizations."

Symptoms.—The most marked and characteristic symptom is the *skin eruption* which may be urticarial as in serum allergy, erythematous, papular, vesicular, hemorrhagic, or a combination of these. Eruptions are especially developed in the allergies to quinin, arsenic, belladonna, copaiba balsam, antipyrin, mercury, salicylicates, and iodids. The same individual may react to the same drug at different times in a different manner. Itching frequently accompanies the rash or may exist without the eruption.

A peculiar form of eruption sometimes found in the allergies to antipyrin, phenacetin, and salipyrin is the "fixed pigmented erythema."

Fever, sometimes preceded by a chill, is one of the most frequent symptoms of drug allergy and may reach as high as 104° to 106° F. It may be present without the skin eruption or the eruption may be present without fever, but as a general rule they are both present.

Edema and especially of the face is frequently found in the allergic reactions to arsphenamin, chloral, antipyrin, opium, and digitalis. *Arthritic and muscular pains* and *adenitis* may develop as in serum disease, as likewise leukopenia and lowering of blood-pressure. Not infrequently the allergic reaction to a drug is unusual and characteristic for a given individual; for example, I know of an individual whose reaction to a minute dose of quinin consisted of a severe balanitis.

Skin reactions are frequently elicited in drug allergies by application of the drug to abrasions, and tests of this kind are employed for diagnostic purposes. They are described in the succeeding chapter.

CHAPTER XXXI

CLINICAL ALLERGY (*Continued*)

ALLERGIC SKIN REACTIONS

The Reactivity of the Skin; Specific and Non-specific Reactions; the Influence of Age, Disease, and Non-specific Factors.—It is highly probable that the skin plays a more important rôle in the mechanism of recovery of disease of the internal organs than is commonly surmised. Certainly the skin and mucous membranes are among the most important factors in natural immunity to microbic infection. As discussed in Chapter IX, the skin may possess an important function in the destruction of microbes and toxic products during elimination, and it is not surprising that the skin and mucous membranes have been found to become sensitized during the course of many diseases, some of which are not cutaneous diseases or characterized by any special cutaneous manifestations. The skin appears to become sensitized or allergic by reason of the intimate part it appears to play in the general processes of infection and immunity.

Allergic skin tests are conducted by applying the agent to abrasions or injecting it very superficially (intradermically). If the cells are sensitized, a local allergic reaction occurs. In addition to this specific reaction, however, a non-specific reaction may occur. Practically any substance injected intracutaneously will elicit a non-specific reaction if the amount injected is sufficiently large.

This non-specific reaction may be due in part to trauma and in part to the production of toxic split products by the ferments of the skin, recently studied by Sexsmith and Petersen.¹ The possibility of its occurrence is so important that in conducting skin tests a control fluid should always be included, if possible, in order to guard against error. Otherwise the amount of allergen applied or injected should be such as is known on the basis of numerous trials with normal individuals, to be less than the amount giving non-specific reactions.

The reactivity of the skin varies with age. Rolly,² working with a variety of bacterial toxins, found that infants did not react, but that reactions occurred with advancing age. Tezner,³ using Witte's peptone, colon bacilli, and tuberculin, observed that at about the time that the skin of children became increasingly sensitive to tuberculin, a corresponding sensitiveness was manifest toward the other substances, a more or less general "sensitization" or "Umstimmung." Sexsmith and Petersen believe that this phenomenon is related to the alteration in the ferments of the skin, the skin of the young containing peptidase in a considerable amount, while the adult skin seems to be without this ferment activity.

The reactivity of the skin may be decreased in cachexia, advanced tuberculosis, pregnancy, and other diseases. From the standpoint of allergy this depression is probably due to desensitization by circulating antigens; from the standpoint of the non-specific or enzyme reaction it may be due to an increase in the titer of the serum anti-ferment. Both processes may be

¹ Jour. Exper. Med., 1918, 27, 273.

² Münch. med. Wchn., 1911, lxiii, 1285.

³ Monatschr. f. Kinderh., 1911, 10, 131.

suppressed by a decrease of permeability of the capillary endothelium, as studied especially by Luithlen, von den Velden, and others.

An increased non-specific reactivity of the skin has been found by Hoke¹ in leukemia and thyroid feeding; Sherrick has found that the administration of potassium iodid has the same effect by accelerating the rate of digestion with the production of split products capable of exciting acute inflammation and commonly resulting in the production of a pustule.

It is apparent, therefore, that the subject of allergic skin reactions is quite complicated and full of possibilities for error. The occurrence of non-specific reactions, differences in the reactivity of the skin according to age, the influence of disease processes in increasing or decreasing reactivity, and the important influence of non-specific agents upon reactivity, are all to be kept in mind when conducting and interpreting skin reactions.

Mechanism of Specific Reactions.—The application of skin and mucous membrane tests for allergy to various substances was given great impetus by von Pirquet's studies with the cutaneous tuberculin test. Since then tests of this kind have been developed for the diagnosis of allergies to other microparasites, serum, pollens, foods, drugs, and other substances, and have generally proved reliable and of considerable diagnostic aid.

The use of these tests is based upon the assumption that in allergy the cells of the skin and mucous membranes share in the sensitization. In tuberculosis this occurs to an exquisite degree; likewise in the majority of cases of hay-fever and other allergies.

When the allergen in suitable form is applied to a mucous membrane, an abrasion of the skin, or injected intradermally, a local reaction of allergic shock is produced. With tuberculin an interval of twelve hours or more usually elapses before a reaction appears, but with pollens and food allergens a reaction usually develops within fifteen minutes. Why there is this difference in the time factor is unknown.

The reaction apparently occurs in or upon the sensitized cells. Cook and Smith² have shown in a conclusive manner by perfusion experiments, employing pieces of excised skin by the Schultz-Dale method, that the antibody occurs in or upon the cells as is apparently true of allergy in general. It is probable that the mechanism of the local reaction is identical with the general reaction and in the nature of an intracellular colloidal phenomenon.

This reaction is expressed clinically by the development of vasomotor changes with hyperemia and edema. The irregularly outlined urticarial-like lesion is the most typical; hyperemia alone is less significant. When the allergen is placed upon the conjunctival or nasal mucous membranes similar changes occur, that is, acute hyperemia, serous exudation, and profuse watery discharge.

Non-specific Skin Reactions.—It is well known that the skins of some individuals are more sensitive than others to trauma of cutaneous tests. This is especially true of persons subject to hay-fever and other allergies. In syphilis the "Unstimmung" of the skin is generally known and may occur in other diseases. An abrasion and application of some ordinarily harmless material, as a drop of decinormal sodium hydroxid solution, may elicit a well-defined wheal. This tendency introduces a source of error and must be checked in every case by a control scarification or injection.

Intradermal injections are especially likely to produce non-specific reactions. These may be due to (a) trauma and the direct injection of an irritant used as a preservative for the material, as phenol, or, to a preformed

¹ Wien. klin. Wchn., 1920, 33, 41.

² Jour. Immunology, 1916, 2, 415.

toxic and irritant substance, as a bacterial toxin; (b) to the production of an irritant by the action of non-specific ferments in the serum or derived from injured cells, upon the protein of the patient's serum, devitalized cells, injected protein, or all three.

According to Petersen the enzymes of the skin vary somewhat according to age. The young skin contains more ereptase (erepsin or peptidase) and little lytic protease; the adult skin, on the other hand, little ereptase and more protease. Neither type of skin contains much antienzyme.

Allergic Skin Reactions as Indices of Infection and Hypersusceptibility.—As is well known, allergic skin reactions may be elicited in various bacterial and protozoan diseases with allergens prepared from the protein of the respective microparasites, particularly in tuberculosis, glanders, typhoid fever, and syphilis. Well-marked and specific reactions have also been found by Amberg¹ and Kolmer and Strickler in ringworm and favus, and isolated reports show that they may be elicited in various other diseases with the proper preparations.

In these conditions, however, the skin reactions are not always elicited, and especially during the early and acute stages. An interval of time is required for the purpose of sensitization, and during the acute stages antigen and antibody may both be present in the cells and body fluids, as shown by Weil² and Denzer,³ with continual interaction expressed in the symptom-complex of the infection, and thereby giving no response when the protein is applied or injected into the skin. Furthermore, the chemical nature of the protein of our allergen may have been altered in the course of preparation to a sufficient extent to fail to elicit an allergic reaction. These and other factors not understood diminish the practical value of a skin test. At present, however, it may be stated that they possess a diagnostic value which is particularly high in chronic infections, and that no other satisfactory clinical or laboratory test for the state of allergy to a particular protein and for the allergic antibody has been discovered except that by which the protein is actually brought into relation with the body cells, as in the parenteral introduction of the protein. Of great interest in this connection is the relation of the intensity of the allergic skin reaction to the extent of the infection. Krause⁴ has recently studied in a thorough and excellent manner the tuberculin skin reaction in relation to experimental tuberculosis, finding that cutaneous hypersensitiveness to tubercle protein is inaugurated by the establishment of infection and the development of the initial focus; that the skin reaction increases with progressive disease; is diminished with healing and increased by reinfection.

The clinical significance and practical value of skin reactions are largely of a diagnostic nature for the detection of hypersensitiveness to a protein or proteins, which may, when introduced into the organism, produce various acute or chronic lesions and symptoms of disease.

Cutaneous Versus Intracutaneous Tests.—Mucous membrane tests are not generally employed except the ophthalmic tuberculin and mallein tests by veterinarians among the lower animals.

Cutaneous tests for allergy were first employed by Blackley in 1873 in hay-fever by the application of pollens to abrasions of the skin. In 1909 Cole conducted a skin test for allergy to buckwheat, but the extensive pioneer work of Schloss since 1912 finally established the value of the cutaneous or "scratch" test for allergic sensitization.

¹ Jour. Exper. Med., 1910, xii, 435. ² Proc. Soc. Exper. Med. and Biol., 1914, xii, 37.

³ Jour. Infect. Dis., 1916, xviii, 631.

⁴ Jour. Med. Research, 1911, xxiv, 361; *ibid.*, 1916, 35, 1, 25.

The intracutaneous test has been developed largely by investigations of Cooke in 1911 and employed and advocated by him up to the present time.

The cutaneous test, consisting in the application of the allergen to a superficial cut or abrasion of the skin, is generally employed, and especially in tests for allergies to pollens and foods. Walker and Adkinson¹ have compared the cutaneous and intracutaneous methods with different proteins in allergic asthmas and found the former specific, sufficiently sensitive, easier to do, and more agreeable to the patient. The intradermic test was found too sensitive and because of the tendency for non-specific reactions, does not always separate closely related proteins; furthermore, it is more difficult to do, causes the patient considerable annoyance and discomfort, and is not as practical when many substances must be tested. In other words, the cutaneous tests while easier, less painful, more prompt in results, and less likely to error by yielding pseudopositive reactions, are less sensitive than the intradermal tests. Therefore, they may fail to indicate an allergy, and when cutaneous tests are negative Blackfan² has recommended that intracutaneous tests are advisable. Due care must be exercised in reading and interpreting the latter to guard against the traumatic and non-specific reactions being regarded as specific allergic reactions.

Brown³ has likewise found the intracutaneous test superior to the cutaneous. In series of 78 patients clinically sensitive to proteins he found positive reactions in all with the intradermal test, 82 per cent. positive with a cutaneous test employing fluid allergens, and only about 50 per cent. positive with the cutaneous test conducted with the dried allergens commonly employed. Brown injects only about 0.01 c.c. which reduces the trauma and tendency to non-specific reactions.

Intracutaneous tests are more likely than cutaneous tests to elicit general reactions by reason of the greater chances for absorption. In cases of extreme sensitization, as allergic asthmas to horse protein, egg-white, and pollens, the cutaneous tests are certainly to be preferred, and if intracutaneous tests are employed, the amounts injected must be very small, as 0.1 c.c. of 1 : 100 or 1 : 1000 dilutions.

Cooke⁴ has studied with particular care these constitutional effects, sometimes following the application of intracutaneous tests and subcutaneous injections for desensitization. He records one death from allergic asphyxia in a child three years of age attributed to skin tests in extreme allergy to glue. Cooke has advised the use of diluted extracts for these tests in order to avoid constitutional effects, warns against frequently repeated tests, and states that not more than six to eight intracutaneous tests should be conducted at one time.

When general symptoms develop after intracutaneous injections, as extensive wheals, erythema, short paroxysmal coughs, or dyspnea, Cooke advises the application of a tourniquet and the injection of adrenalin 1 : 1000 in dose of 1 c.c. for adults and 0.4 to 0.6 c.c. for children. Also the intravenous injection of 0.001 gm. strophanthin for adults if there is cardiac dilatation.

In veterinary practice cutaneous tests are not employed, preference being expressed for the intradermal tests employing the skin at the root of the tail, the nose, or mucosa of the eye.

Technic of the Cutaneous Test.—The skin of the arm or forearm is cleansed with alcohol and dried. Abrasions are made for each substance to be tested, with one or two extra for controls. Walker makes a series of linear

¹ Jour. Med. Research, 1917-18, 37, 287.

² Amer. Jour. Med. Sci., 1920, 160, 341.

³ Jour. Immunology, 1922, 7, 97.

⁴ Jour. Immunology, 1922, 7, 119.

cuts about $\frac{1}{8}$ inch long (never longer than $\frac{1}{4}$ inch) with a sharp scalpel on the flexor surface of the forearm. These cuts are only deep enough to penetrate the outer layers of the skin, care being taken not to draw blood. Abrasions may also be made with a needle as commonly practised in cowpox vaccination; the von Pirquet skin bore is also very useful (Fig. 160). Care must be exercised against drawing blood; a slight oozing of serum is desirable. The instrument should be wiped with alcohol.

The cuts or abrasions should be at least $\frac{1}{2}$ inch apart in order that positive reactions may be clearly defined; when only a few tests are to be applied, they should be 1 inch or more apart (Fig. 154).

When ten or more tests are to be conducted at one time, Walker's method is very good, as two rows of incisions are easily accommodated on the forearm.

On each cut or abrasion is placed a different protein. If the protein is in powder form a minute amount is placed on the cut, followed by the addition of 1 drop of N/20 sodium hydroxid to dissolve the protein and hasten



FIG. 154.—METHOD OF MAKING SCARIFICATIONS FOR CUTANEOUS ALLERGIC TESTS WITH DALAND LANCET.

its absorption. It is well to rub the protein in for a few seconds with an applicator needle or scarifier, but care should be exercised against causing bleeding. I use small round wooden applicators like toothpicks (Fig. 155). After making the abrasions, a toothpick is dipped into N/10 sodium hydroxid solution and applied to a cut which transfers sufficient of this solution; the powder is next applied and rubbed in for a few seconds with the same toothpick, which is then discarded and a fresh one used for each substance to be tested.

The control receives a drop of hydroxid solution, followed by the same amount of rubbing in exactly the same manner except that no protein is used. If hydroxid is employed in the tests, it is essential to use it in the control, as some skins are quite sensitive to this substance.

One-half hour later the proteins are washed off and the results noted. If the protein causes a marked reaction which may be accompanied by considerable itching, it is usually removed before a half-hour in order to avoid discomfort and the absorption of too much of the protein.

As a general rule pure proteins are employed, but with individuals who are extremely hypersensitive due care must be exercised against the production of general reactions by the application of too much protein. In practice this refers especially to allergic asthmas to horse protein and eggs. In these cases the first tests may be conducted with 1 : 10 or 1 : 100 dilutions of horse-serum and egg-white; if reactions do not result, undiluted allergens may be employed.

The Cutaneous Reactions.—In reading the results the control is first inspected and the other reactions compared with it. Sometimes the control shows the presence of a small wheal surrounded by erythema, and in this case reactions with proteins are interpreted as positive when larger and better defined.

The reactions vary considerably with different proteins and with different individuals. Walker has described four main types, as follows, with proteins of bacteria, pollens, hair, etc.:



FIG. 155.—METHOD OF APPLYING ALLERGENS TO CUTANEOUS ABRASIONS IN ALLERGIC SKIN TESTS

(a) An elevated white urticarial wheal which measures about 1 cm. in diameter and is surrounded by a pinkish, red areola.

(b) A smaller wheal, but a larger area of erythema which often measures 1 or 2 cm. in diameter; this reaction looks not unlike a large mosquito bite which has been irritated by scratching.

(c) A large area of erythema with little or no elevation about the cut.

(d) A doubtful reaction consisting of a small area of erythema measuring $\frac{1}{2}$ cm. or less in diameter. If the control shows no reaction or much less than this degree of erythema, this reaction may be regarded as doubtful or weakly positive and should be repeated at a later date.

A fifth type of reaction is described by Walker and occasionally seen. At the end of a half-hour it is negative or nearly so, but next day the skin about the cut is hot, very red, and slightly elevated. The cut may contain pus which has always proved to be sterile. This delayed reaction may not be allergic and its significance is doubtful.



FIG 156 —POSITIVE ALLERGIC REACTIONS TO POLLENS

Itching and a burning sensation may accompany these reactions. A negative reaction is one that shows practically no difference from the control cut or abrasion.

As a general rule the reactions begin to fade after a half-hour, but may persist for two or three hours. The cuts or abrasions heal rapidly and without infection, but it is advisable to keep them clean and free of gross contamination.

The cutaneous tuberculin reactions are described later; they develop much more slowly and present a different appearance in that a wheal or urticarial lesion is not usually produced.



FIG. 157.—METHOD OF ADMINISTERING AN INTRADERMAL INJECTION

The skin has been cleansed with alcohol and pinched up between the thumb and index-finger of the left hand; the needle (No. 26) has been entered into the epidermis and 0.1 c.c. of fluid injected. Note the anemic area, indicating that the injection has been intradermic.

Technic of Intracutaneous Tests.—The skin of the outer side of the arm or flexor surface of the forearm is generally employed and should be cleansed with alcohol.

The injections are made with a 1 c.c. perfectly working syringe graduated into 0.1 or 0.01 c.c. amounts and fitted with a fine needle. I prefer the Record or Luer syringe graduated in 0.1 c.c. and fitted with a No. 26 needle. The syringe and needle should be sterilized beforehand and must be thoroughly cleansed for each protein. For this reason a series of tests requires considerable time.

The injections are given intracutaneously and not subcutaneously. The skin is pinched up between the forefinger and thumb and the needle entered eye upward until the eye is completely in the epidermal layer of the skin (Fig. 157). The injection is accompanied by the production of a whitish

elevated area (Fig. 158); if this is not seen, the injection is too deep and apt to be unsatisfactory. A slight stinging hot pain is experienced.

Not more than 0.2 c.c. should be injected; it is better to inject less, as 0.1 c.c. or even 0.05 c.c., as these reduce the degree of trauma and pain. The solution of protein should not be more than 1 or 2 per cent; in cases of extreme sensitiveness, as in allergic asthmas due to pollens, serum or foods, the dilution should be less, for example, as 0.1 per cent. (1 : 1000) and only 0.05 c.c. injected in order to avoid general reactions.

When the tests are conducted with bacteria isolated from the sputum in asthma or from some other focus of infection, I inject 0.1 c.c. of a suspension in saline solution of approximately 500,000,000 per cubic centimeter.



FIG. 158.— SHOWING THE SMALL ANEMIC AREA IMMEDIATELY AFTER AN INTRACUTANEOUS INJECTION.

The injections should be at least 2 inches apart. By starting near the shoulder a series of six to eight injections can be readily given an adult on one side.

A control injection is always advisable. Since the allergens are generally dissolved in saline solution and preserved with 0.2 to 0.3 per cent. phenol or tricesol, I inject 0.1 c.c. of a control fluid of sterile saline solution containing 0.25 per cent. of the same preservative as the solutions of allergens.

Intracutaneous Reactions.—Within a few minutes after the injection of the protein solution an area of edema and erythema develops similar to the positive cutaneous reactions. The control and negatively reacting sites also show some erythema, but as a general rule the true positive reactions

are readily differentiated from these in fifteen to thirty minutes by a larger area of erythema and a wheal-like lesion.

Positive reactions usually persist for twenty-four to forty-eight hours as a papule with slight edema and erythema. As a rule the traumatic and non-specific protein reactions subside in this time and permit more accurate readings to be made.

The Effect of Drugs Upon Skin Reactions.—It is now generally recognized that *the oral administration of iodids and bromids may influence intracutaneous skin reactions to the extent of increasing their degree and severity or producing well-marked papular or pustular reactions in the skins of persons who did not react to these injections in preliminary tests, and who are not allergic.*

Ambery and Knox¹ found that the intravenous administration of neutral sodium ortho-iodoxybenzoate and sodium iodosobenzoate inhibited the development of reactions following the intracutaneous injection of serum in sensitized rabbits; sodium iodobenzoate and sodium benzoate in equal molecular concentrations had no inhibitory effect on the local reaction. The authors believed that the inhibitory influence of these drugs was attributable to their effect on oxidative processes. Opposite results have been reported by Sherrick,² who found that *the administration of potassium iodid either simultaneously or shortly before or after the intracutaneous injection of luetin and agar, resulted in the production of pustular or nodular reactions in healthy non-syphilitic persons.* Kolmer, Matsunami, and Broadwell³ confirmed these observations among healthy non-syphilitic persons, persons sick with diseases other than syphilis, and normal rabbits and guinea-pigs. The oral administration of potassium iodid may cause the site of a former intracutaneous injection of luetin or agar to "light up" and present well-defined inflammatory changes sometimes leading to pustulation as pointed out by Sherrick; we also found that a purer form of luetin, prepared of washed spirochetes and free of all culture-media, was influenced in a much less degree by potassium iodid.

In a further study of this subject by Kolmer, Immerman, Matsunami, and Montgomery,⁴ the influence of iodids, bromids, chlorids, ether, and chloroform upon cutaneous and intracutaneous tests with luetin, tuberculin, and other substances were studied.

Potassium iodid administered orally in amounts of 10 gm. per day for two or three days was found to have the most influence upon skin reactions; the bromids exerted a similar but less marked influence, and the chlorids almost none. Ether and chloroform anesthetics exerted no demonstrable effects.

Intracutaneous tests were mostly influenced; cutaneous tests were sometimes influenced and particularly by potassium iodid, while conjunctival tests were not influenced at all.

Lyons,⁵ Cole and Paryzek⁶ have also noted the influence of potassium iodid upon intracutaneous reactions with luetin. Farr⁷ has noted an apparently similar influence upon the luetin reaction by mercury administered by inunction.

I am of the opinion that the influence of these drugs upon skin reaction by increasing their severity and a tendency to papulation and pustulation is exerted upon the traumatic and non-specific phases. It would appear probable that the iodids, bromids, and to a less extent the chlorids, are cap-

¹ Jour. Amer. Med. Assoc., 1912, lix, 1598.

² Jour. Amer. Med. Assoc., 1915, lxxv, 404.

³ Jour. Amer. Med. Assoc., 1916, lxxvii, 718.

⁴ Jour. Lab. and Clin. Med., 1917, 2, 401.

⁵ Southern Med. Jour., 1916, 9, 483.

⁶ Jour. Amer. Med. Assoc., 1917, 68, 1089.

⁷ Jour. Amer. Med. Assoc., 1915, 64, 850.

able of increasing tryptic activity by removal of antiferment, after the hypothesis of Jobling and Petersen,¹ which is followed by the increased local digestion of proteins by the tissue proteases with the production of diffusible irritants.

At any rate it is well for the physician to keep in mind the influence of these drugs upon intracutaneous reactions, and especially the influence of potassium iodid upon the luetin reaction, in order to avoid erroneous results.

SKIN TESTS FOR SERUM ALLERGY

If the individual is known or suspected to be extremely sensitive to the serum, cutaneous tests should be done; otherwise the intracutaneous test is more sensitive and to be preferred when serum is to be given intravenously for therapeutic purposes.

For conducting the *cutaneous test* either raw normal or immune serum (not purified or concentrated antitoxins) or dried serum proteins may be employed. Walker² prefers the latter, which is prepared as follows:

The proteins are precipitated by adding to the serum several volumes of acetone and centrifuging. The precipitate is well mixed with absolute alcohol, centrifugalized, and the alcohol decanted; this process is repeated. The protein is then washed twice with ether in the same manner. The material is then dried and the powder employed for cutaneous tests according to the technic previously described.

Reactions develop within fifteen minutes as described; Fig. 159 shows the urticarial-like lesion that develops on the arm of one of my colleagues following scarification and the thorough application of a drop of horse-serum. This man is also susceptible, to a less extent, to guinea-pig- and rabbit-serum, and is seized with sneezing and distressing dyspnea after entering a house where these animals are kept.

For *intracutaneous tests* sterile raw normal or immune serum (not purified or concentrated serum) should be diluted 1 : 50 with sterile saline solution and 0.1 c.c. (= 0.002 c.c. undiluted serum) injected intracutaneously; or, the dose injected may be larger, as 0.1 c.c. of 1 : 5 dilution (0.02 c.c. undiluted serum), if only a slight degree of hypersensitiveness is suspected. A small white wheal showing the little depressions of the hair-follicles is produced. An injection of 0.1 c.c. salt solution is made as a control.

If the test is negative the wheals produced by both serum and saline solution disappear in a few minutes. If sensitiveness exists, a genuine urticarial wheal develops in five to ten minutes, which reaches its maximum within one hour and fades away within a few hours. General symptoms of flushing of the face, tachycardia, and difficult breathing develop, but rarely and only in cases of extreme hypersensitiveness.

SKIN TESTS FOR POLLEN (HAY-FEVER) AND OTHER PLANT ALLERGIES

Cutaneous tests are now employed almost solely, the conjunctival, nasal, and intracutaneous tests formerly used being practically abandoned.

The allergens are prepared of a very large variety of pollens and grasses by different manufacturing laboratories and are available for the profession in convenient and generally satisfactory outfits.

The pollens are mechanically isolated and macerated in 10 per cent. sodium chlorid solution, which has been rendered faintly alkaline with sodium hydroxid. The filtered extract is dialyzed until free from salts and

¹ Jour. Exper. Med., 1914, 19, 480.

² Jour. Med. Research, 1916-17, 35, 498.



FIG. 159.—LOCAL SERUM ANAPHYLACTIC REACTIONS.

Dr. W. P., anaphylactic reactions fifteen minutes after application of serum; rabbit-serum in the upper; horse-serum in the lower. Subject to asthmatic attacks when in an animal house or stable where rabbits, horses, and guinea-pigs are kept.

the dialyzate precipitated with acetone and the pollen protein thus obtained dried with anhydrous acetone. The products are odorless and tasteless powders, insoluble in water or dilute acids, but readily soluble in physiologic salt solution and dilute alkalies. Wodehouse¹ has described a simple method employing carbontetrachlorid, which was found more rapid and to yield larger amounts of pollen.

Coca² has recently described methods for the collection of various pollens and prepares extracts as follows: The dried pollen is repeatedly extracted with three volumes of ether until the decanted ether shows only a slight yellow color. Four or five extractions suffice for ragweed pollens; fewer for grass pollens because they contain less oil. The ether is finally removed by immersing the beaker in hot water (50 to 60° C.). Each gram of pollen is now extracted with 300 c.c. of the following fluid:

Sodium chlorid.....0.5 per cent.

Sodium bicarbonate in such concentration that 10 c.c. of

the final fluid equals about 3 c.c. of $\frac{n}{10}$ alkali.

Carbolic acid in final concentration.....0.4 “

The mixture is covered with toluol and extraction continued for four days at room temperature, the sediment being shaken up once or twice daily. The supernatant fluid is now decanted and filtered through a sterile Berkefeld filter. Each cubic centimeter usually contains about 0.3 to 0.4 mgm. of nitrogen.

In spring hay-fever, perennial vasomotor rhinitis, and vernal conjunctivitis it is usually necessary to test with many different allergens (see list on page 664). In autumnal hay-fever tests with goldenrod and ragweed usually suffice.

The tests are conducted as previously described and the results usually read within half an hour. Figure 156 shows the appearance of positive and negative reactions.

Similar tests may be conducted for the allergies to rhus (dermatitis venenata), although the history is usually so definite that these tests are not required unless there is doubt regarding the kind of plant to which the patient is hypersensitive, as poison ivy, poison oak, or poison sumac.

SKIN TESTS FOR ALLERGIES TO HAIRS, DANDRUFFS, AND FEATHERS

These are conducted by the cutaneous method. The allergens are available as prepared by different manufacturing biologic laboratories. These tests are especially useful in the diagnosis of the allergic asthmas and perennial vasomotor rhinitis. The history of the patient usually suggests the allergens, but if not, a series prepared of the hairs and dandruffs of the horse, cat, dog, and other domestic animals should be employed as well as allergens of goose and chicken feathers.

For preliminary tests allergens of hairs and dandruffs may be prepared, according to Walker, by placing a handful of uncleaned hair or a few grams of dandruff in a small bottle, cover with 14 per cent. alcohol and extract the proteins, for several days, with frequent shaking, followed by filtration. A drop of this alcohol extract is used in the skin test. Purified alkaline and acid metaproteins from the hair of the cat and dog and dandruff of the horse may be employed, the immochemistry and preparation of these being described by Wodehouse.³

¹ Boston Med. and Surg. Jour., 1916, clxxiv, 430.

² Jour. Immunology, 1922, 7, 166. ³ Jour. Immunology, 1917, 2, 227, 237, 243.

Since the yield of protein powders is usually very small, Walker prepares them in 1 : 100 dilution in N/100 sodium hydroxid or weak acid or alcohol, as necessity may require in each particular protein, and standard dilutions are made from this stock solution, as 1 : 1000, 1 : 10,000, 1 : 100,000, and 1 : 1,000,000. These various dilutions are used for skin tests, so that it is possible to determine the degree of sensitiveness. The dry undiluted proteins are kept in stock, from which these dilutions are made as required.

Coca¹ has described a method for preparing extracts of *dandruff*, *hair*, *wool*, and *feathers* which is essentially as follows: The oils are removed by mixing the material with three volumes of ether and after an hour or two the ether is decanted and discarded and the last traces completely removed by immersing the beaker in water at 50° to 60° C. Each gram of dried material is now mixed with 10 c.c. of toluol and then with 100 c.c. of the sodium bicarbonate extracting fluid described above for the extraction of pollens. The mixture is stoppered and extracted for twenty-four hours followed by centrifuging or paper filtration. This fluid is then passed through a sterile Berkefeld filter and usually contains about 0.5 mgm. of nitrogen per cubic centimeter.

SKIN TESTS FOR FOOD ALLERGIES

These are generally conducted by the cutaneous method, but as previously stated, the intracutaneous method is more delicate, but likewise more subject to error in interpretation. Probably a good rule for practice is to first conduct cutaneous tests, and if the results are negative, intracutaneous tests may be performed.

A very large number of allergens of vegetables, grains, fruits, meats, fishes, and other animal products, as cow's milk and eggs, are now available in convenient form for these tests, being prepared by different manufacturing firms. Allergens of foods eaten after cooking may be prepared of these heated foods; this eliminates the heat-coagulable proteins unless they happened to be partly hydrolyzed by the heating. Methods for the preparation of allergens of vegetable and animal foods have been fully described by Wodehouse and Olmstead.² Shorter methods consist in the removal of vegetable or animal fats if any are present by extraction of the ground-material with petroleum ether or centrifuging in the case of milk, extracting with faintly alkalinized 10 per cent. solution of sodium hydroxid, removing the salts by dialyzation and precipitating the proteins with acetone, and drying into powders with anhydrous acetone.

Coca³ has described a method for preparing food allergens which is essentially as follows: *Dried materials*, as *whole wheat flour*, *rice meal*, *ground beans*, etc., are weighed, and each kilogram thoroughly mixed with 150 c.c. of toluol and then with 2200 c.c. of the sodium bicarbonate extracting fluid described on p. 681 for the extraction of pollens. Extraction is continued at room temperature for twenty-four hours, being thoroughly shaken up at least once in this period, when the fluid is decanted and set aside. The material is now extracted again for twenty-four hours with 2000 c.c. of extracting fluid followed by decantation. The two fluids are now mixed, covered with toluol, and set aside for a few days for the collection of any sediment that may form. The supernatant fluid is now decanted or the sediment removed by centrifuging or paper filtration, the material being finally passed through a sterile Berkefeld filter.

¹ Jour. Immunology, 1922, 7, 166.

² Boston Med. and Surg. Jour., 1916, clxxvi, 467, 468; *ibid.*, 1917, clxxvii, 85.

³ Jour. Immunology, 1922, 7, 166.

Moist materials, from which little or no juice can be expressed, such as *meats, sweet potatoes, green pea, turnip, cauliflower, string bean, celery, cabbage, lettuce, spinach, cucumber, potatoes, lima bean, etc.*, are passed twice through a meat chopper, mixed with extracting fluid, and covered with a thin layer of toluol. The extraction is generally interrupted after twenty-four hours and the fluid is obtained by pressing the mixture in a stout towel by hand. The amount of extracting fluid varies with the nitrogen content. For the meat three or four volumes of the fluid (figured on the weight of the material) are used. For the vegetables, one or two volumes.

Some of the *shell fish* yield a juice in addition to the meat. To the juice one-quarter volume of the following "preserving fluid" is added:

Sodium chlorid	2.5 per cent.
Sodium bicarbonate	1.5 "
Carbolic acid in final concentration	2.0 "

The meat is mixed with three or four volumes of the extracting fluid previously described and the two mixtures thrown together, covered with toluol and extracted for at least two weeks. The material is now decanted, filtered through paper, and finally a sterile Berkefeld filter.

With such fruits and vegetables that can be peeled, as *peach, tomato, orange, lemon, and grape-fruil*, it is advantageous to make separate extractions of juice squeezed from the pulp and the ground up peelings. The juice may be extracted with one-quarter volume of "preserving fluid" and the ground up pulp and peelings with two or three volumes of "extracting fluid." The two mixtures may now be thrown together, covered with toluol, extracted for two weeks at room temperature, and finally filtered through paper and sterile Berkefeld filters.

Milk *casein* is prepared by adding acetic acid to milk and collecting the precipitate in a towel. This material is now washed by immersing the towel in several changes of water. The casein is now forced through a wire kitchen strainer, while kept moistened with distilled water. To this suspension is now slowly added 4 per cent. sodium hydroxid solution until nearly all of the casein is dissolved. Stirring is continued some time beyond this point, and if some casein still remains undissolved the solution will be neutral to litmus. The mixture is now filtered through paper and the filtrate kept under toluol. Before filtration through a Berkefeld filter the solution is further diluted with 0.4 per cent. carbolic acid in 1 per cent. sodium chlorid to a nitrogen content of 2 mgm. per cubic centimeter. After filtration the nitrogen content per cubic centimeter is further reduced to 1 mgm.

From the original acetic acid filtrates the other proteins may be precipitated by adding $1\frac{1}{2}$ volumes of saturated ammonium sulphate and the precipitate collected on paper freed of the ammonium salt by dialysis against changes of 1 per cent. sodium chlorid.

Skin tests for allergy to eggs may be conducted by rubbing a little egg white and yolk into abrasions or cuts; if intracutaneous tests are conducted the amount injected may be 0.1 c.c. of a 1 : 100 dilution.

Intracutaneous tests are conducted by dissolving the dried proteins in physiologic saline solution; usually the injection of 0.1 c.c. of a 1 : 100 dilution is sufficient.

SKIN TESTS FOR ALLERGIES TO DRUGS

These are usually conducted by the cutaneous method. In tests for allergy to quinin both Boerner¹ and Malley and Richey² have observed almost immediate reactions following the application of 1 or 2 drops of a 10 per cent. solution of quinin bisulphate to abrasions. In allergies to procain, Lane³ has elicited reactions by the application of a 2 per cent. solution and shows the appearance of positive reactions. The tests may be conducted by adding a minute amount of the drug to an abrasion and dissolving in the exuding serum aided by the addition of 1 drop of saline solution. A control with saline solution should always be included.

Tests for allergies to arsphenamin, mercury, and other metals may be conducted in the same manner. With the vegetable drugs, as belladonna, stramonium, etc., fluidextracts may be employed. Extracts are readily prepared by macerating the leaves and extracting with 14 per cent. alcohol for several days, the tests being conducted by applying 1 drop of the extract to an abrasion of the skin.

If intracutaneous tests are conducted, the amount injected should not be greater than 0.1 c.c. of 1 : 100 dilution.

SKIN TESTS FOR ALLERGIES TO BACTERIA, FUNGI, AND PROTOZOA

In Allergic Asthma, Bronchitis, and Rhinitis.—Cutaneous or intracutaneous tests may be conducted. Stock allergens of different bacteria are available in dried powder form for cutaneous tests in exactly the same manner as the hay-fever tests. I prefer intracutaneous tests conducted with separate autogenous antigens prepared of the different bacteria recovered from cultures of the sputum, nose, or other focus.

Stock dried allergens are prepared by Walker⁴ by cultivating the bacteria on an appropriate medium and securing them in a sediment by removing the growths with saline solution containing 0.5 per cent. phenol and centrifuging. The sediment is now thoroughly mixed with phenolized saline solution, centrifugalized, and the saline decanted. The sediment is now mixed with absolute alcohol containing 0.5 per cent. phenol, centrifugalized, and the alcohol decanted. This process is repeated once more with alcohol, twice with ether, and the sediment dried into a powder. The method of conducting the tests has been described.

For intracutaneous tests with autogenous antigens I proceed as follows:

1. Cultures are made on Petri plates of hormone blood-agar and the different bacteria isolated and identified.
2. Pure cultures of these are grown on appropriate solid media for twenty-four to forty-eight hours and removed with sufficient saline solution to give dense suspensions of at least 2,000,000,000 per cubic centimeter, or the bacteria may be grown in a fluid medium and secured by centrifugalization. If any medium has been carried into the suspension it is filtered through sterile paper and the bacteria are washed once with saline solution containing 0.5 per cent. phenol before being resuspended in the saline solution.
3. Each suspension is heated in a water-bath at 60° C. for one hour, cultured for sterility, and preserved with sufficient phenol to make 0.5 per cent.
4. For the skin tests a small amount of each suspension is diluted with sterile saline solution to give approximately 500,000,000 per cubic centi-

¹ Jour. Amer. Med. Assoc., 1917, lxviii, 907.

² Arch. Int. Med., 1919, 24, 378.

³ Arch. Dermat. and Syph., 1921, 3, 235.

⁴ Jour. Med. Research, 1916-17, 35, 488.

meter; 0.1 c.c. of each is injected intracutaneously. A control injection of saline solution is made at the same time.

5. The reactions are read approximately one and twenty-four hours later.

6. Those bacteria causing positive reactions are then incorporated into an autogenous vaccine prepared from the stock suspensions.

As a general rule the bacteria recovered from cases of rhinitis, bronchitis, and asthma are of the following: staphylococci (albus and aureus), streptococci (hemolytic and non-hemolytic), pneumococci (usually Type IV), Micrococcus catarrhalis, and Bacillus pseudodiphtheria. Tests are not conducted with the fungi and yeasts. Great care must be exercised in conducting these intracutaneous tests with bacteria, as some, and especially *M. catarrhalis*, are irritating and likely to produce "positive reactions" in nearly every case (Rackemann). For this reason Rackemann prefers injecting 0.1 to 0.25 c.c. of the bacterial suspensions (1,000,000,000 per cubic centimeter) *subcutaneously* and selecting for treatment those producing local reactions.

Allergic Skin Tests in Focal Infections and Mixed Infections.—I have also used this technic for determining whether or not sensitization exists to bacteria recovered from the apical abscesses, tonsillar cultures and other foci in relation to focal infection; also with the different bacteria recovered in cultures from chronic suppurative lesions, as otitis media. Organisms producing positive reactions are incorporated into autogenous vaccines for treatment.

TUBERCULIN REACTION

An account of Koch's discovery of tuberculin in 1891 is given in the chapter on Tuberculin Therapy. Suffice it to say here that Koch was most interested in the curative properties of tuberculin, and while he has accurately and clearly described the classic picture of the systematic tuberculin reaction, he failed to appreciate the true significance of the reaction at the site of injection, although its occurrence is carefully noted.

The Tuberculin Reaction.—The reaction to tuberculin is characterized by three essential features:

1. A constitutional reaction, consisting of fever and the accompanying general symptoms of lassitude, anorexia, and rapid pulse, varying in severity with the intensity of the reaction.

2. A local reaction at the site of administration, varying in intensity from slight tenderness and redness to severe inflammation with adenitis.

3. A focal reaction about the tuberculous lesion.

These reactions do not by any means run parallel. An intense local reaction may occur, with no or but slight constitutional disturbance. Not infrequently, and particularly in slight pulmonary lesions, signs indicating a focal reaction may not be elicited.

Nature of the Tuberculin Reaction.—Koch believed that the tuberculin reaction was due to a summation of the effects of the injected toxin and the toxic bodies formed by the tubercle bacillus within the infected host. Koehler and Westphal in 1891 suggested that, by a union of the tuberculin with the products of the tubercle bacillus, a third new body was formed in the tuberculous focus. Marmorek in 1894 suggested that the tuberculin stimulated the tubercle bacilli to secrete a fever-producing substance.

Finally, in 1903 von Pirquet and Schick explained the reaction as due to a "vital antibody reaction," and this explanation is the one most generally accepted today. Tuberculin is not toxic for non-tuberculous individuals

and produces no reactions upon cutaneous application, intracutaneous or subcutaneous injection. In tuberculous individuals, however, surprisingly small amounts produce marked reactions. The local reaction on the skin at the point of application or injection and the focal reaction of hyperemia and exudation of the tissues around the foci of disease are regarded as allergic reactions. The general symptoms of fever, tachycardia, lassitude, etc., are due to the absorption of toxic substances from the foci of disease, according to Kraus,¹ absorption being stimulated and facilitated by the focal allergic shock. Furthermore, there is increased capillary permeability due in part to the non-specific phase (see Chapter XXXIX) which results in an outpouring of plasma and enzymes and enhanced proteolytic digestion and production of toxic substances.

The focal tuberculin reaction is primarily an allergic reaction. Evidently the phenomenon is one of cellular allergy, the antibody being in or upon the cells and the reaction being an intracellular colloidal phenomenon. Evidence of the cellular allergic nature of the reaction has been produced by Weil² and more recently by Smith,³ with the Schultz-Dale method employing the uteri of sensitized guinea-pigs. As discussed in a previous chapter, the humoral theory of anaphylaxis is inadequate for explaining the reaction on the basis of the production of a poison (anaphylatoxin) by either specific amboceptors and complement or by a ferment; one millionth of a cubic centimeter of Koch's old tuberculin may elicit a reaction in the tuberculous and it is impossible to understand how sufficient anaphylatoxin could be produced from so small an amount.

In addition to being an allergic phenomenon the focal tuberculin reaction is probably also in part non-specific due to the stimulation of cells (plasma-activation of Weichardt) by proteins in the tuberculin. Furthermore, the injection of other non-specific agents as vaccines and proteoses intravenously and milk intramuscularly, may elicit reactions around foci of tuberculosis analogous to those engendered by tuberculin. These reactions are regarded as due to stimulation of cellular activity with increased capillary and lymphatic permeability and exudation of serum, leukocytes, enzymes, etc. It is to be emphasized, however, that in comparison to tuberculin relatively large amounts of these non-specific agents are required to elicit focal reactions. Autogenous sputum vaccines are especially likely to produce these reactions.

How sensitization of the skin, mucous membranes, and other organs of the body to tuberculin is brought about in tuberculosis is not known; experimental attempts to sensitize the skin of normal animals with injections of tuberculin have usually failed, although the skin has been sensitized by injections of the proteins of tubercle bacilli. The chemical nature of O. T. is not definitely known, but it probably contains proteoses and sensitization of animals with proteoses is generally weak and irregular. It is highly probable that the sensitinogen is a product of living bacilli in the tissues or a new combination substance with the tissues, inasmuch as sensitization can only be produced regularly by foci of active tuberculosis.

Specificity of the Tuberculin Reaction.—The tuberculin reaction is highly specific. This does not mean that every case of tuberculosis will give a tuberculin reaction, and positive reactions are occasionally found in apparently healthy persons and cattle. Furthermore, the focal tuberculin reaction may be partly non-specific and capable of being engendered by non-

¹ Jour. Med. Research, 1916, 35, 43.

² Jour. Amer. Med. Assoc., 1917, 68, 972.

³ Jour. Immunology, 1922, 7, 47.

specific agents, as previously mentioned. The conditions under which a negative reaction may occur in the presence of tuberculosis are, however, fairly well understood, and physicians should be thoroughly acquainted with these. Likewise most instances in which a positive reaction was observed in the apparent absence of tuberculosis have usually narrowed down to the fact that the lesion was so small or so situated as to escape detection, and, indeed, this has been shown so conclusively by autopsies that, in the presence of a tuberculin reaction, on the autopsy must rest the burden of proof and blame. When we realize how small a lesion may produce hypersensitiveness, it will readily be understood how easily the clinician and pathologist may fail to detect the lesion.

A large part of our knowledge regarding the specificity of the tuberculin reaction has been gained from veterinary practice, as the results of a test in an animal could immediately be controlled by the autopsy findings. Thus Fraenkel¹ collected from the literature 8000 carefully observed instances, and found only from 2 to 3 per cent. of differences between the result of the tuberculin test and of the autopsy. Voges,² in 7327 instances, noted 2.7 per cent. of contradictions. Kuhnau,³ Bang,⁴ and von Behring⁵ speak of similar experiences.

It has long been known that the prevalence of tuberculous findings anatomically far exceeded the number of cases recognized clinically. Among cattle, anatomic tuberculosis is found in from 12 to 25 per cent., and about 80 per cent. or more react to tuberculin. In many of the latter, however, the disease does not progress, but, on the contrary, tends to recede.

Similar conditions exist in human pathology. That tuberculosis is very frequent among adults is now well known. The figures of Nageli⁶ and Burkhardt⁷ showed that the increasing frequency of tuberculous infection with advancing years reached over 90 per cent. among those past the eighteenth year; these figures are now well corroborated. Hamburger,⁸ in the published results of an analysis of 848 autopsies on children, showed that tuberculosis was in evidence in 40 per cent., increasing from 4 per cent. among infants under three months of age to 70 per cent. among children from eleven to fourteen years. This explains, in part at least, the relatively high resistance of children to tuberculin, the difficulty there is said to be in eliciting reactions, and the necessity that exists for using large doses. Usually, when children fail to react, it is because they are not tuberculous or because the lesion is too small, whereas in later years, until adult life is reached, the reaction is observed with increasing frequency and with smaller doses, because the incidence of infection increases progressively from 5 per cent. during infancy to 90 per cent. and over in adult life.

The prevalence of tuberculosis, however, by no means indicates that the infected individual suffers ill health or will succumb to the infection. An individual may be enjoying excellent health, and still harbor a tuberculous lesion, and display a marked degree of hypersensitiveness to tuberculin. Such a person is not usually regarded as tuberculous until there are tangible symptoms referable to its existence. *It is important to remember that tuberculin is an index of tuberculous infection, and not of disease in a clinical*

¹ Zeitschr. f. Tuberk., 1900, i, 291.

² Tuberculin und Organismus, Jena, 1905, 77.

³ Berl. tierarztl. Wchn., 1899, 78.

⁴ Sixth International Congress on Tuberculosis, 1908, 211.

⁵ Beit. z. exper. Therap., 1905, x, 1.

⁶ Virch. Arch. f. path. Anat., 1900, clx, 426.

⁷ Zeitschr. f. Hyg. u. Infectiousk., 1906, liii, 139.

⁸ Wien. klin. Wchn., 1907, xx, 1070.

sense. Numbers of persons and cattle reacting to tuberculin remain healthy and do not develop symptoms of disease, the autopsy disclosing the presence of inactive or regressing lesions.

In former years it was considered possible to obtain false positive reactions in convalescents and patients in an enfeebled condition who were non-tuberculous, and also in other diseases, such as syphilis, leprosy, and actinomycosis. More accurate anatomic statistics and careful studies of the tuberculin test administered to a large number of individuals, healthy, tuberculous, and sufferers from other diseases, have gradually changed the attitude of the profession and served to establish the high specificity of the tuberculin reaction.

Sources of Error in the Tuberculin Reaction.—From the foregoing it will readily be understood that most errors in the tuberculin reaction refer to false negative rather than to false positive reactions.

False positive reactions may be observed in leprosy, where the bacillus bears such close morphologic and biologic resemblance to the tubercle bacillus, and it is likewise true that massive doses of tuberculin injected subcutaneously may produce a toxic fever in debilitated individuals, but positive reactions in healthy individuals can usually be ascribed—(a) to a small hidden tuberculous lesion or (b) to a healed tuberculous lesion. As just stated, tuberculin simply indicates hypersensitiveness to the tubercle protein, and this may exist with a very small unimportant lesion, or persist after a lesion has been “healed” to the extent of encapsulation.

False negative reactions are much more likely to occur, and the various conditions that may be responsible for these should be well understood and remembered.

1. In the final stage of tuberculosis, especially in miliary tuberculosis and in tuberculous cachexia, as in the third stage of pulmonary tuberculosis, the tuberculin reaction may be negative, or be attained only after the injection of very large doses. There is a lessened cutaneous reactivity (cachectic reaction), marked by the appearance of colorless or pinkish spots, instead of an intense papillary eruption. Koch and Ehrlich have explained this by assuming that the tissues had become too thoroughly saturated with tuberculin produced at the infected area to respond to further artificial additions. This condition may be regarded as analogous to a state of anti-anaphylaxis in which we may consider the free and sessile receptors united with the tubercle protein or the cells loaded with the antigen, with depression of cellular activity.

2. In the first stage of infection. At this period the antibody has not been formed in sufficient amounts, different authors obtaining such findings in nurslings.

3. In small, completely healed lesions, especially in those showing nothing but scar tissue, as in the apex of a lung. In these the antibody and cellular hypersensitiveness have disappeared, and while the lesion may have been tuberculous, one cannot tell anatomically, in a given instance, whether or not hypersensitiveness should have been present.

4. During continued treatment with tuberculin, when the reaction may be negative owing to a condition of anti-anaphylaxis.

5. During measles. As von Pirquet and Preisich have demonstrated, the cutaneous reactivity disappears during the first days after the eruption, reappearing after about a week. Greuner showed that the *Stichreaktion* which occurs after large doses of tuberculin did not disappear entirely, indicating that in measles there is a lessened activity rather than a total disappearance.

6. Finally, according to von Pirquet, there are a few cases in which we have a minimal reactivity and to which none of the former explanations can be applied. Some cases of active tuberculosis may show only a slight hypersensitiveness, although they are not cachectic.

Of course, it can readily be understood that the use of weak or an otherwise unsatisfactory solution of tuberculin and an improper dosage and technic will greatly influence the results. Likewise errors in interpreting what constitutes a positive tuberculin reaction are to be guarded against. This applies especially to veterinary practice. For example, cattle brought from the fields and confined in a stall for the purpose of making a tuberculin test may exhibit a fever for several days without apparent cause. On the other hand, dishonest dealers may force cattle to drink cold water or have given cold water irrigations just before the temperature is taken, preventing the registration of a febrile reaction.

Methods of Conducting the Tuberculin Test.—The object of the tuberculin test is to introduce sufficient tubercle protein to react with the tubercular antibody, which shows its presence and effects by a general, a local, or a focal reaction, or by a combination of these. Various methods have been proposed, of which the following are best known:

1. The *subcutaneous tuberculin test* is the oldest test of its kind, having been discovered by Koch¹ in 1891. It consists in the subcutaneous injection of old tuberculin. A positive reaction manifests itself in a constitutional disturbance, accompanied by fever, a local reaction at the site of injection ("Stichreaktion"), and frequently a focal reaction at the site of tuberculous disease.

2. The *cutaneous tuberculin test* of von Pirquet,² consisting in the local application of old tuberculin to a superficial abrasion of the skin. A positive reaction is indicated by redness, edema, and other inflammatory phenomena.

3. The *conjunctival tuberculin test* of Wolff-Eisner³ and Calmette,⁴ consisting in the local application to the conjunctiva of one eye of a drop of 1 per cent. solution of old tuberculin or purified tuberculin. A positive reaction is indicated by congestion and lacrimation.

4. The *percutaneous tuberculin test* of Moro and Doganoff,⁵ consisting in the application of tuberculin ointment prepared by mixing equal parts of old tuberculin and anhydrous lanolin and applying it to the skin over the upper portion of the abdomen or about the nipple. A positive reaction is indicated by an efflorescence of papules upon the anointed skin.

Analogous to this test is that of *Ligniere*, who rubs the skin vigorously with alcohol and xylol until slightly reddened. After drying, 5 or 6 drops of undiluted tuberculin are applied and rubbed in with a rubber-coated finger for one or two minutes.

5. The *intracutaneous tuberculin test* of Mendel⁶ and Mantoux,⁷ consisting in injecting into the superficial layers of the skin 0.05 c.c. of diluted old tuberculin. A positive reaction is denoted by infiltration and hyperemia about the site of injection, similar to the reaction to the cutaneous test.

Comparative Delicacy and Relation of the Various Tuberculin Tests.—In judging of the comparative delicacy of the various tuberculin tests by a review of the literature, it must be remembered that results will vary accord-

¹ Deut. med. Wchn., 1891, xvii, 101.

² Berl. klin. Wchn., 1907, xlv, 699.

³ Discussion, Berl. klin. Wchn., 1907, xlv, 70.

⁴ Presse médicale, 1907, xv, 388.

⁵ Wien. klin. Wchn., xx, 933.

⁶ Med. Klin., 1908, iv, 402.

⁷ Münch. med. Wchn., 1908, No. 40.

ing to the portion of the body inoculated, as sensitiveness of the cells varies in different parts of the body, and there are individual differences in various persons that are difficult or impossible to explain.

By comparing the figures obtained as the result of different tests upon the same person with the relative frequency of individual tests, Hamman and Wolman¹ have drawn the following conclusions:

"1. The intracutaneous and subcutaneous local tests are the most delicate we possess. They reveal practically the full percentage of tuberculosis-infected individuals.

"2. In the order of their sensitiveness, the tests arrange themselves as follows:

- Intracutaneous Test.
- Subcutaneous Local Test.
- Cutaneous Test.
- Subcutaneous Test.
- Percutaneous Test.
- Conjunctival Test.

"3. There is a definite but not a constant relation between the various tests. An individual reacting to the conjunctival test will, as a rule, give all the others, but not always. The cutaneous or the subcutaneous tests may be negative when the conjunctival is positive. The subcutaneous positive when the cutaneous is negative, etc. Some of the unusual variations may, no doubt, depend upon faulty technic in performing the tests, but all can certainly not be thus explained. Local changes in sensitiveness and variation in the facility of absorption are probably factors, but the exact conditions are not understood.

"4. We have been unsuccessful in an attempt to make the cutaneous test with different strengths of tuberculin equivalent to the conjunctival test."

Although the subcutaneous test may be dangerous on account of the harm that may result from too severe focal reactions, yet, when carefully conducted, it is frequently the method of choice, especially in the diagnosis of an obscure pulmonary lesion, and in bone, joint, skin, and other local infections, where the focal reaction may be detected by direct examination. It is to be emphasized, however, that the absence of focal changes during a constitutional reaction does not exclude the tuberculous nature of a suspected lesion. In children, as shown by Hamill, Carpenter, and Cope,² the various tuberculin reactions are likely to yield results that are quite similar.

The Value of Tuberculin in Diagnosis.—*As previously stated, a reaction to tuberculin means essentially that the individual reacting has a tuberculous infection, and in itself means nothing more.* Since tuberculin tests disclose inactive and relatively benign tuberculous infections, it has little value, in doubtful cases, in aiding us to decide whether or not the individual has active disease, which clinically is the type of infection about which we are most concerned. Lack of critical discernment in the interpretation of the reaction and its apparent indefiniteness have contributed toward diminishing its diagnostic value.

A positive tuberculin reaction is to be regarded as a symptom, or as another link in the chain of clinical evidence, but is not in itself indisputable evidence that a certain lesion is tuberculous, for it can never decide with certainty an otherwise doubtful diagnosis. A similar example is that of a positive Wassermann reaction in a patient with a lesion in the throat; such a reaction does

¹ Tuberculin in Diagnosis and Treatment, 1912, Appleton & Co., 167.

² Archiv. Int. Medicine, 1908, ii, 405.

not necessarily mean that the lesion is syphilitic, for the lesion itself may be cancerous, although, coincidentally, a latent syphilitic infection may be present.

If tuberculin could differentiate between active and inactive lesions according to the degree of reaction, its value would be greatly increased. While the studies of Krompecker and Romer upon animals indicate that the more virulent the infection the greater the degree of hypersensitiveness, no such fixed relation exists in man. All that may be said is that, in general, the severer the reaction, the more acute the infection. On the other hand, acute miliary tuberculosis or chronic cachectic cases may react negatively.

The conditions under which a negative reaction may be obtained in a tuberculous person are to be carefully borne in mind, for if these can be excluded, a negative tuberculin reaction precludes, in all probability, an active or clinically important tuberculous lesion. Tuberculin has, therefore, a higher negative than a positive value in diagnosis.

While it is obviously beyond the scope of this volume to discuss the diagnostic value of tuberculin in tuberculous infection of the different organs, I may briefly refer to a few of the more important conclusions reached by individual investigators of large experience in this particular field:

1. *In the diagnosis of pulmonary tuberculosis, while a positive constitutional or local tuberculin reaction is never conclusive evidence that a definite pulmonary lesion is tuberculous, a focal reaction, on the other hand, tells definitely of the presence of the disease, and shows, in some measure at least, its extent. In these questionable cases, therefore, the subcutaneous injection of tuberculin finds its most important application, since a definite focal is of more value than a local reaction.*

Tuberculin may also be of service when the symptoms suggest the presence of a pulmonary tuberculous lesion, but the physical signs are indefinite. Here the focal reaction is likely to be slight and to escape detection, so that one of the cutaneous tests are usually employed.

2. *In the diagnosis of bone, joint, and glandular tuberculosis the subcutaneous test is likely to be most valuable, on account of the focal reaction of hyperemia, swelling, heat, and pain about the lesions. The cutaneous test is also valuable, but since this may react on account of the presence of a lesion situated elsewhere, the focal reaction is more conclusive. According to Hamman and Wolman, (a) a focal reaction confirms the diagnosis of tuberculous bone or joint disease; (b) an absence of reaction to the subcutaneous test excludes, with the highest probability, the presence of tuberculosis.*

3. *In the diagnosis of genito-urinary and pelvic tuberculosis a positive tuberculin reaction is of little value unless the subcutaneous method is employed and the physician is sure of his ability to detect a focal reaction, a proceeding that may be very difficult or indeed impossible. A positive cutaneous test indicates the presence of a lesion somewhere in the body, without disclosing the nature of the renal or pelvic lesion. A negative reaction, however, is of more value, especially when the physician bears in mind the conditions under which a falsely negative result may occur.*

4. *In the diagnosis of tuberculosis of the eye, ear, and larynx the tuberculin reaction usually has a limited value, because the nature of the disease can be so readily detected by direct inspection. In tuberculosis of the larynx a focal reaction may be dangerous on account of edema. Similarly in advanced tuberculosis of the ear a focal reaction may lead to extension of the process to the meninges. In these instances, therefore, a cutaneous test should first be made, and if it is found to be negative or inconclusive, the subcutaneous test should be applied with extra caution.*

5. *In the diagnosis of tuberculosis of the skin* tuberculin may occasionally prove of value—especially the focal reaction following subcutaneous injection or a direct local application upon the lesion with a weak dilution such as a 1 per cent. solution of old tuberculin.

6. *In the diagnosis of tuberculosis of a serous membrane* tuberculin usually possesses a limited value. In *tuberculous meningitis* the subcutaneous test is contraindicated, as a focal reaction may do harm. Owing to the acute infection the cutaneous test may be negative, and even if positive, would not aid greatly in the diagnosis because the meningeal condition is always secondary to a primary focus. *Tuberculous pleurises*, dry or with effusion, and unaccompanied by evident pulmonary disease, are frequently associated with a low-grade tuberculin hypersensitiveness. According to Hamman and Wolman, in a large proportion of cases of pleurisy with effusion the conjunctival test is negative and the cutaneous test but mildly positive. Bandler and Roepke assert that in a dry pleurisy increased pain and more pronounced and extensive friction may occur during a constitutional reaction to the subcutaneous test and indicate a focal reaction. In *tuberculous peritonitis* the tuberculin test is frequently negative. A positive reaction has far more value, especially in virulent types of the disease, which come on insidiously with little or no constitutional disturbance.

The Value of Tuberculin in Prognosis.—As previously stated, tuberculin may not react in the very early and very late cases of tuberculosis. In patients with rapidly advancing lesions the power to react tends to decrease and frequently is absent. But this condition is apparent without the aid of tuberculin. While Wolff-Eisner and Stadelmann¹ laid some stress upon the conjunctival reaction in prognosis, others have been unable to confirm the results, and, as stated by Hamman and Wolman,² tuberculin fails to yield us information of prognostic value that other methods of clinical observation do not bestow.

The Dangers of Tuberculin.—Practically, the only danger lies in the subcutaneous and conjunctival tests. With the subcutaneous test, the greatest danger in pulmonary tuberculosis is the possibility of overdosage, with the production of an extensive focal reaction which may bring on hemorrhage or lead to local extension of the lesion. Because of its very important bearing on tuberculin treatment, the subject will be discussed in the next chapter. The same danger of excessive focal reaction holds for tuberculous meningitis (increased intracranial pressure), in tuberculous laryngitis (edema), and in tuberculosis of the ear and nasal accessory sinuses (extension to meninges).

The cutaneous, intracutaneous, and percutaneous reactions are practically devoid of danger, providing that a careful technic is observed.

Regarding the dangers of the conjunctival test, opinions differ. There can be no doubt, however, but that distressing sequelæ, such as severe recurring conjunctivitis, phlyctenular conjunctivitis, and corneal ulcers with permanent opacities have resulted from the use of this test. Most of the unfavorable results have followed instillation in already diseased eyes, or of too strong solutions, but this is not true of all cases. As will be pointed out further on, in the first instillation not over 1 per cent. of old tuberculin should be used, and a second instillation should never be made in the same eye for at least several years.

Since old people are especially prone to conjunctival inflammation and corneal ulceration, it is probably better to exclude them from the test.

¹ Deutsch. med. Wchn., 1908, xxxiv, 180.

² Tuberculin in Diagnosis and Treatment, 1912, 179.

Another drawback to this test is the possibility of a "flare up" in the eye following subsequent subcutaneous administration of tuberculin, either for diagnostic or therapeutic purposes. Hamman and Wolman, however, do not consider this dangerous, and have observed but 2 cases in an extensive experience.

THE SUBCUTANEOUS TUBERCULIN REACTION

As has been stated repeatedly, the subcutaneous injection of tuberculin is resorted to at the present time mainly for the purpose of eliciting a focal reaction, and, as a rule, this is more easily appreciable when the general reaction is well marked. *If tuberculin is used simply to establish whether or not a person is hypersensitive, this fact may be demonstrated by employing much smaller doses, as by the cutaneous, intracutaneous, or conjunctival tests, the patient being spared the discomfort of the constitutional symptoms.*

Variety of Tuberculin.—Koch's old tuberculin is now used almost exclusively. The technic of its preparation is given in the chapter on Tuberculin Therapy.

Manufacturing chemists usually market this tuberculin in a series of dilutions, labeled and accompanied by explicit directions, so that the physician may administer practically any dose desired by injecting so many minims of such or such dilution. Otherwise a series of dilutions are readily prepared in the physician's office or dispensary, according to the method followed by Hamman and Wolman:

(a) Seven wide-mouthed bottles of about from 12 to 15 c.c. capacity, and fitted with ground-glass or rubber stoppers, are sterilized, labeled, and numbered from 2 to 8.

(b) The diluent is sterile 0.8 per cent. salt solution with 0.25 per cent. pure phenol. This is readily prepared by adding 8 gm. of pure sodium chlorid and 2.5 c.c. of pure phenol to 1000 c.c. of distilled water. Dissolve, and filter into one large Erlenmeyer flask or, preferably, into ten smaller flasks. Sterilize in the Arnold sterilizer for one hour, or in the autoclave for twenty minutes, or by gently boiling for fifteen minutes on each of two consecutive days.

(c) Into each bottle place 9 c.c. of the diluent with a graduated and sterile pipet. Bottle 1 contains pure tuberculin. To bottle 2 add 1 c.c. of tuberculin and shake carefully; to bottle 3, 1 c.c. from bottle 2 and shake; to bottle 4, 1 c.c. from bottle 3 and shake; continue in this manner to bottle 8, from which 1 c.c. is discarded.

(d) We now have the following dilutions:

No. 1—pure tuberculin.							
No. 2—0.1	c.c.	tuberculin in each cubic centimeter	(100	mg. tuberculin.)			
No. 3—0.01	c.c.	" " " "	(10	" "			
No. 4—0.001	c.c.	" " " "	(1	" "			
No. 5—0.0001	c.c.	" " " "	(0.1	" "			
No. 6—0.00001	c.c.	" " " "	(0.01	" "			
No. 7—0.000001	c.c.	" " " "	(0.001	" "			
No. 8—0.0000001	c.c.	" " " "	(0.0001	" "			

(e) These dilutions are usually prepared every two weeks. When not in use, the bottles are kept in a cool, dark place. It may not be necessary to prepare all dilutions. For example, dilutions Nos. 3 and 4 are sufficient for diagnostic purposes, as 0.1 c.c. of No. 4 equals 0.1 mg. of tuberculin and 1 c.c. of No. 3 equals 10 mg., thus affording an ample range of dosage.

Method of Conducting the Test.—1. The patient's temperature and pulse-rate should be taken every two hours for from four to seven days.

This is easily accomplished in a hospital; ambulatory patients can usually be readily trained to take their own temperature. All observations should be recorded in writing, and preferably on a temperature chart. The patient's temperature must be constantly below 99° F. before beginning the test, and, if necessary, prolonged rest in bed should be enforced to overcome any existing fever. The test may be given in spite of a daily rise of not over 100° F., but tuberculin by subcutaneous injection should be given only exceptionally to febrile patients.

2. A very careful physical examination should be made, and the results recorded just before and just after the test in order to detect a focal reaction. This is extremely important, for it is our main justification for injecting the tuberculin.

3. Injections are made subcutaneously in the region of the back, below the angle of the scapula, or in the arm. The skin needs no preparation other than to be rubbed with alcohol. The injections are best given during the late evening hours or early in the morning, in order that the temperature and pulse changes may be observed, especially twelve hours after the injection. Records of temperature and pulse should be made every two hours during the day and night for forty-eight hours following an injection. Hamman and Wolman recommend the "Tuberculin Sub 2 Syringe," made by the Randall, Faichney Co. Each syringe should be sterilized by boiling prior to use, and it is recommended that a separate syringe be provided for each dilution.

4. Considerable controversy has arisen over the size of the doses to be employed. Koch's directions called for 1 mgm. at first, then for 5, then for 10, and if no reaction followed this dose, it was repeated. There is a decided tendency, however, to use smaller doses. If the object is to establish the presence or absence of tuberculin hypersensitiveness, mild reactions will suffice, and for this purpose small doses repeated or in gradually increasing amounts may be given. If one aims to produce a focal reaction, larger doses and more rapid increase are desirable. Hamman and Wolman advise the following plan for adults: For the first dose, $\frac{1}{3}$ mg. is given. If there is a slight febrile reaction of about one-half a degree, and especially if this is accompanied by a local reaction at the point of injection, the second dose, which is the same as the first, is given at the end of forty-eight hours. The reaction will now most likely be more conclusive. If there is no appreciable reaction after the first dose, the second, consisting of 1 mg., is given at the end of forty-eight hours, and the third dose, if one is necessary, consists of 5 mg. Occasionally the third dose must be repeated, or even 10 mgm. given if the negative result is at variance with the clinical impressions.

If the temperature shows any irregularities, the intervals between injections should be prolonged to three or four days or more.

Roth-Schultz advise the following doses: 0.5 mg.; 1.25 mg., and 2.5 mg. as the terminal dose.

For children under fifteen years of age smaller doses are indicated—an initial dose of 0.1 mg. and a terminal dose of 1 mg., with one or two intervening doses. Baldwin has advised 0.05, 0.2, 0.5, and 1 mg.

The Reaction.—The *constitutional reaction* is quite variable. Fever is the most delicate indicator. A rise of 1° F. or more above the previous maximum is considered positive, especially if it is accompanied by a local and a focal reaction. A definite febrile reaction due to tuberculin is rare without the presence of a local reaction to the same or the preceding doses. General symptoms of headache, muscle pains, anorexia, nausea, etc., may accompany the reactions. The *local reaction* consists of redness and pain at

the site of injection, with tenderness of the neighboring lymph-glands, and is absolutely specific of tuberculin hypersensitiveness. The *focal reaction* consists of an inflammatory reaction with the production of râles, change in breath sounds, etc.

THE INTRACUTANEOUS TUBERCULIN REACTION

Variety of Tuberculin.—Koch's old tuberculin is used either in one dose of 0.005 mg., or preferably in three different doses injected simultaneously; these will be described further on.

Method of Conducting the Test.—The skin of the forearm is cleansed with alcohol and then dried. A small glass syringe fitted with a fine needle is used. A separate syringe is used for the control fluid, consisting of sterile salt solution, and three others for each of the different dilutions used. In performing this test Hamman and Wolman make four simultaneous injections:

First: 0.05 c.c. of sterile normal salt solution (control).

Second: 0.05 c.c. of a 1 : 1,000,000 dilution of old tuberculin, which equals 0.00005 mg. This equals a dose of 0.05 c.c. of dilution No. 4, just described in the preceding test.

Third: 0.05 c.c. of a 1 : 100,000 dilution, or 0.05 c.c. of dilution No. 3, equivalent to 0.0005 mg.

Fourth: 0.05 c.c. of a 1 : 10,000 dilution, or 0.05 c.c. of dilution No. 2, equivalent to 0.0005 mg.

Mantoux uses the last or fourth dose only. The injections are given with the skin held taut or pinched up in a fold between the index-finger and thumb. The needle is inserted superficially, with the aperture directed toward the outer surface of the skin. If the point of the needle is in the skin, a white elevation occurs immediately upon the introduction of the solution; if it is in the subcutaneous tissue, no infiltration is apparent. Cohen¹ injects T. R., the first doses being one ten-millionth, one millionth, and one hundred thousandth of a milligram. If these injections provoke no reactions, he repeats the tests with doses ranging as high as 10 mg.

The **reaction** consists of infiltration and hyperemia about the site of injection similar to the reaction in the cutaneous test. It appears in from six to eight hours, reaches its maximum intensity in from twenty-four to forty-eight hours, and usually disappears in from six to ten days. The salt solution generally produces a traumatic reaction, similar to a mild tuberculin reaction, which subsides in forty-eight hours.

The reactions are best recorded after twenty-four hours, and the simplest method of recording the results is to measure the width of the area of infiltration of each reacting point.

Wildbolz Intracutaneous Urine Test.—Wildbolz² reports that his research during the last year and a half and experiences with more than 200 persons have demonstrated that when there is an active process of tuberculosis the urine contains an antigen which injected by the Mantoux intradermal technic induces infiltration and redness. According to Wildbolz this does not occur with urine from healthy persons or in urine from persons with healed tuberculous processes. It is said never to occur unless the person gives a positive response to injection of 1 : 10,000 tuberculin, but it seems to occur whether the urine is from the person being tested or not, so

¹ Jour. Infect. Dis., 1917, xx, 233.

² Corresp.-Blatt. f. Schw. Aertze, 1919, 49, 793.

long as he has an active tuberculous process anywhere in the body, in glands, peritoneum, lung, bones, or elsewhere. Wildbolz evaporates morning urine to 1 : 10 passed once or twice through a paper filter impregnated with 2 per cent. phenol, and then makes three sets of two injections on the arm, the two upper with 1 : 1000 tuberculin; 3 or 4 cm. below this, two with 1 : 10,000 tuberculin, and, the same distance below, two with a minute amount of the 1 : 10 evaporated urine. The response with an active tuberculous process is the same with the urine as with the diluted tuberculin, but the tuberculin response persists unmodified after the process has healed, while the urine response fades out completely. A similar response was never obtained in the non-tuberculous, not even in syphilis, influenza, etc., with the single exception that urine containing large amounts of staphylococci induced a reaction, so that the findings are not pathognomonic in certain cases of nephritis. With this exception, this biologic reaction, Wildbolz states, may be depended on to reveal the tuberculous or non-tuberculous nature of lesions, and will also disclose when they are healed. If the urine reaction persists after the clinical healing of the known process we can be confident that there is some other active process elsewhere.

The specific nature of the urine reaction is demonstrated still more conclusively by the fact that, after subsidence of the urine reaction, if an injection of 1 : 1000 tuberculin is made nearby, the apparently extinct urine reaction flares up anew, the infiltration and redness becoming distinct again.

Eliasberg and Schiff,¹ Imhof,² Schmidt,³ and Gibson and Carrol⁴ have reported favorably upon this test; the latter states that the salt content of the urine may modify the reaction and that the urine should be made free of salts before being applied.

THE CUTANEOUS TUBERCULIN REACTION (VON PIRQUET)

Variety of Tuberculin.—Undiluted old tuberculin is now used almost exclusively in conducting the test.

Method of Conducting the Test.—1. The flexor surface of the forearm is chiefly used for making the applications, but it should be remembered that tests performed on different portions of the body are not strictly comparable.

2. The skin is cleansed lightly with alcohol and dried. Three abrasions are made, about 1½ or 2 inches apart, with a von Pirquet skin borer (Fig. 160) or with a needle, small lancet, or blood sticker. The object is to scarify the superficial layers of the skin, avoiding as much as possible bleeding, although a few small points of blood should appear. To the upper and lower abrasions add 1 drop of tuberculin; after ten minutes wipe away the excess with a bit of cotton. No shield or protective dressings are required. The middle abrasion is the control, and shows the amount of traumatic reaction following the scarifying process. Due precautions should, of course, be observed that none of the tuberculin flows down the arm and reaches this spot.

3. The tests are inspected at the end of twenty-four hours.

The Reaction.—The traumatic reaction as shown in the control area may present an inflammatory areola with, at times, slight infiltration. Before a test may be considered positive its areola should be at least 5 mm. wider than the control area. The reactions are usually designated as follows:

¹ Monatschr. f. Kinderh., 1920, 19, 5.

² Schweiz. med. Wchn., 1920, 50, 1033.

³ Schweiz. med. Wchn., 1921, 51, 996.

⁴ Jour. Amer. Med. Assoc., 1921, 76, 1381.



FIG. 161.—A POSITIVE CUTANEOUS TUBERCULIN REACTION (VON PIRQUET).
Child with incipient pulmonary tuberculosis; a + reaction. The control scarification is barely to be seen, and is midway between the tuberculin reactions.

1. *Negative Reaction*.—No appreciable difference between the tuberculin areas and the control.
2. *Slight Reaction*.—Definite but slight redness with some infiltration.
3. *+ Reaction*.—A wider area of redness, with definitely raised centers.
4. *++ Reaction*.—Wider area of redness, with more marked infiltration than + (Fig. 161).
5. *+++ Reaction*.—Unusual redness and a wide area of infiltration, all cases which go on to vesiculation.

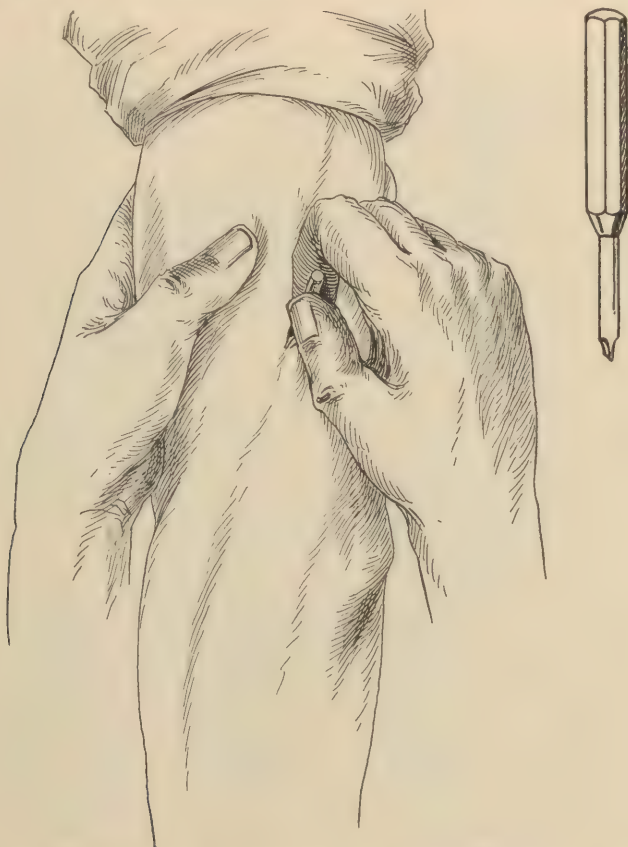


FIG. 160.—METHOD OF PERFORMING A VON PIRQUET TUBERCULIN TEST.

The abrasion is being made over the insertion of the deltoid muscle. The borer is held firmly and perpendicular to the arm. A quick rotatory motion serves to remove a circular area of epidermis. The *borer* is shown in the upper right-hand corner.

The usual or normal reaction begins to appear in from four to six hours, reaches its maximum intensity in from twenty-four to forty-eight hours, and then fades rapidly, although the infiltration may persist for some days. Special types of the reaction have been described as follows:

1. The *premature reaction*, characterized by a rapid course and slight intensity. This type is supposed to occur in patients with manifest tuberculosis who are not doing well.
2. The *persisting reaction*, which reaches its maximum intensity about the second day and persists for a week or longer.

3. The *late reaction*, which appears after twenty-four hours and develops and recedes slowly. These last two types are believed to occur in patients having inactive lesions.

4. The *cachectic reaction*, which is characterized by infiltration with little or no redness. This type is common in the late stages of tuberculosis.

5. The *scrofulous reaction*, which is characterized by numerous small elevated nodules, which may also appear on the extremities and trunk. This reaction is peculiar to children and rare in adults.

THE CONJUNCTIVAL TUBERCULIN REACTION (CALMETTE)

Variety of Tuberculin Used.—A 1 per cent. solution of Koch's old tuberculin is now generally used, as it is quite reliable, least expensive, and the results that follow its use are more regular than those obtained with purified tuberculin (0.5 to 2 per cent. aqueous solution).

Method of Conducting the Test.—The conjunctivæ of both eyes are inspected to ascertain if there is any evidence of disease and if they are strictly comparable in color. The lower lid of one eye is drawn forward to form a little pouch, and the patient is directed to look upward; 1 drop of a 1 per cent. dilution of old tuberculin is then applied from an ordinary eye-dropper to the lid at the inner canthus. Profuse lacrimation impairs the test, and it is useless to attempt it with weeping or resisting children. If no reaction is apparent and it is still desired to further the test, 1 drop of a 5 per cent. dilution may be placed in the opposite eye.

The Reaction.—In a positive reaction the conjunctiva begins to redden in from six to eight hours, and reaches its maximum in from twenty-four to forty-eight hours, and then rapidly subsides and disappears in from four to six days. In mild reactions the inner canthus is the seat of the most marked changes. Positive reactions have been classified as follows:

+ Reaction: Definite palpebral redness.

++ Reaction: More marked palpebral redness with secretion.

+++ Reaction: Palpebral and bulbar redness with subjective symptoms and well-marked secretion. (See Fig. 162).

Precautions.—On account of severe reactions and the danger of inflicting permanent injury on the cornea there is a growing tendency to regard this reaction with disfavor. Hamman and Wolman, however, believe that, with proper precautions, these risks may be minimized, if not completely avoided; they believe, moreover, that the risk incurred is not great enough to warrant the abandonment of a procedure that has proved itself of such great value in diagnosis. Nevertheless, they emphasize the necessity for observing proper precautions, and give the following rules to be adopted:

1. The conjunctival test should never be repeated in the same eye.

2. A solution stronger than 1 per cent. original tuberculin should never be used for making the first instillation.

3. Any existing inflammatory disease of the eye is an absolute contra-indication to the test.

4. The test should not be given to manifestly scrofulous children.

5. Skin diseases in which the lesions are situated upon the face, near the eye, especially when these are suspected of being tuberculous, preclude the application of the test.

6. It is safest not to give the test to elderly persons, and particularly to arteriosclerotics, as they are unduly prone to develop corneal ulceration.



FIG. 162.—A POSITIVE CONJUNCTIVAL TUBERCULIN REACTION (WOLFF-EISNER-CALMETTE).
Severe reaction (+ + +) in a tuberculous cow; appearance of the eye about fourteen hours after second instillation of tuberculin.



FIG. 163.—A POSITIVE PERCUTANEOUS TUBERCULIN REACTION (MORO).
E. McK., adult male with arrested early pulmonary tuberculosis. Lesions about sixty hours after application of tuberculin ointment.

THE PERCUTANEOUS TUBERCULIN REACTION (MORO)

Variety of Tuberculin Used.—This consists of 5 c.c. of old tuberculin and 5 grams of anhydrous lanolin thoroughly mixed. The ointment retains its potency for many months when preserved in a cold dark place. Manufacturers market the preparation in small collapsible tubes containing sufficient for at least one or two tests.

Method of Conducting the Test.—A piece of ointment about the size of a pea is *thoroughly* rubbed for at least a minute into an area of skin about 2 inches in diameter over the upper abdomen or near a nipple. The patient may be instructed how to apply this ointment, or the physician may do it himself, protecting the finger with a rubber cot.

The Reaction.—This may appear within twenty-four hours, or be delayed for as long as from four to six days. It consists of an eruption of slightly elevated papules situated upon a hyperemic base, which vary in size from a pinhead to large areas of infiltration (Fig. 163). It subsides in from three to ten days. Moro described these grades of reaction as follows:

1. *Mild reactions:* From 1 to 10 scattered papules.

2. *Moderate reactions:* About 50 or more papules, partly discrete, partly confluent, and with a general reddening of the skin. There may be well-marked itching.

3. *Severe reactions:* Numerous large, extremely red papules or vesicles up to 8 mm. in diameter, having an intensely reddened base.

The **cutaneous test of Ligniere** is conducted by washing the skin with alcohol to produce redness; 6 drops of Koch's old tuberculin is then applied and rubbed into the skin by the protected fingers. The reaction appears in twenty-four to forty-eight hours and is quite similar in appearance and interpretation to the Moro reaction.

STREPTOTRICHIN REACTION

Among the diseases in man and animals that are produced by the streptotriches may be mentioned madura foot, actinomycosis, nocardiosis, and pseudotuberculosis. These diseases are, as a rule, characterized as slow, progressive, localized processes which are, however, capable of an acute general pyemic or pneumonic course. Claypole¹ has shown that they run from mycelial, non-acid-fast forms through non-acid-fast or partially acid-fast bacillary forms to strongly acid-fast forms that are closely allied to the tubercle bacillus. Not only are these gradual transitions in type demonstrable from one species to another, but they may actually occur in the cultivation of a given species. A given organism may from time to time be more or less acid-fast and bacillary, or again at one time almost wholly mycelial and later bacillary.

In a considerable number of human cases of cervical adenitis and pulmonary disease that clinically are indistinguishable from tuberculosis some form of streptothrix has been found in pure culture by Flexner,² Lubarsch,³ Ophüls,⁴ Burnet,⁵ and Foulerton.⁶ That a distinct though unknown percentage of glandular, pulmonary, and bone tuberculosis, so-called, is due to infection with a streptothrix is becoming evident from the work of Bridge⁷ and Claypole. Even with the examination of discharges and sputum the diagnosis is overlooked. In the presence of a non-acid-fast streptothrix the

¹ Jour. Exper. Med., 1913, 17, 99.

² Jour. Exper. Med., 1898, 3, 435.

³ Ztschr. f. Hyg., 1899, 31, 185.

⁴ Jour. Med. Research, 1902, 8, 242.

⁵ Compt. rend. Soc. de biol., 1913, lxxiv, 674.

⁶ Lancet, 1899, 2, 779; *ibid.*, 1913, 1, 381.

⁷ Jour. Amer. Med. Assoc., 1911, lvii, 1501.

carbolfuchsin stain results in the report of "no tubercle bacilli found." On the other hand, an acid-fast streptothrix, particularly if non-mycelial, is passed as *Bacillus tuberculosis*.

Claypole¹ prepared solutions from two types of streptothrix, in all respects similar to old tuberculin (concentrated glycerin-bouillon growths). Her cases were tested by the skin test of von Pirquet with old tuberculin, with Streptothricin H. (Streptotricin Hominis, mycelial, non-acid-fast), and with Streptothricin E. (*S. eppingeri* bacillar, partly acid fast).

In 45 control cases which gave no clinical evidence of tuberculosis, 22 reacted to old tuberculin, but none to either of the streptotrichins. In 55 cases of suspected tuberculosis, comprising 42 lung, 11 gland, and 5 bone involvements, 37 reacted to tuberculin (67 per cent.); 13 of these cases reacted to Streptothricin H.; 8 to Streptothricin E., and 6 gave reactions to two of the test solutions.

Of the 13 Streptothricin H. reactions, 11 were lung cases. In 9 of these no tubercle bacilli were found, but in all of them thread-like, Gram-positive organisms resembling Streptothrix H., in the 2 others acid-fast bacilli were found as well as the mycelial organisms, and one reacted to tuberculin and one to Streptothricin E. (acid-fast), as well as to Streptothricin H. The other two positive Streptothricin H. cases were glandular; both were negative to tuberculin. In one of these cases Gram-positive segments and rods were found histologically.

Eight cases reacted to streptothricin E., 6 with lung involvements and 2 with bone lesions; 3 of the lung cases were negative to tuberculin and had no tubercle bacilli in the sputum, without doubt infections with a less acid-fast organism than *Bacillus tuberculosis*. Two of the 3 remaining cases reacted to tuberculin and to streptothricin E. In one of these tubercle bacilli were found in the sputum. The sixth case reacted to both streptotrichins and had a mycelial Gram organism and an acid-fast organism in the sputum. The bone cases did not react to tuberculin and the infections were by inference due to the Streptothrix eppingeri group of organisms.

The data from these cases indicates that the streptothricin reaction may be a means of differential diagnosis between mycelial streptothrix infection, partly acid-fast streptothrix infections, and tuberculosis.

TUBERCULIN REACTIONS AMONG THE LOWER ANIMALS

In veterinary practice tuberculin is used almost solely for diagnostic and only occasionally for therapeutic purposes.

Four reactions are in common use:

The Subcutaneous Test.

The Conjunctival Test.

The Cutaneous Test.

The Intracutaneous Test.

The technic for conducting these tests and the reactions secured are quite similar to those just described, and I would refer the veterinary surgeon to these respective descriptions. Differences in technic are confined principally to dosage.

The Subcutaneous Tuberculin Test.—This is conducted with Koch's old tuberculin. The method of preparation is given in the succeeding chapter, under Tuberculin Therapy. *Concentrated tuberculin* is prepared by concentrating the bouillon filtrate to one-tenth its original volume. *Diluted tuberculin* is the concentrated product diluted to its original volume.

¹ Arch. Int. Med., 1914, 14, 104.

by mixing 1 part of the dilution with 9 parts of 0.5 to 1 per cent. phenol in sterile normal salt solution or distilled water.

The injections are always given subcutaneously in some convenient area, preferably around the shoulder, which has been shaved and cleaned beforehand with a solution of creolin.

Cary¹ has recently described the following method:

"1. Secure the cattle in separate stalls or by chains, ropes or stanchions, so as to confine them in fixed places, and then number, tag, or mark them for temporary or permanent identification.

"2. Take preinjection temperatures two hours apart, at least three times. Better take preinjection temperatures once every two hours, from 6 A. M. to 6 or 10 P. M.

"3. In all normal animals inject 3 to 10 c.c. of standard subcutaneous tuberculin at 6 or 10 P. M.

"4. Begin taking postinjection temperatures not later than eight hours after time of injection. For animals that have been repeatedly tested or have previously been given large doses of tuberculin it is better to begin taking temperatures not later than four hours after injection (for early reactors continue the regular two-hour temperatures until the eighteenth or twentieth hour after injection. When no regular reaction appears and the temperature rises to 104° F. or higher at the sixteenth or eighteenth hour postinjection, continue taking temperatures up to the twenty-fourth or twenty-eighth hour (for late reactors).

"5. Give the same care, feed, and water both days, and avoid sudden changes of any kind. At no time give heavy feeds, large quantities of water, or produce excitement or exercise.

"6. Interpret the clearly, carefully, and accurately recorded records as follows:

"(a) A regular or typical reactor is one where the postinjection temperatures make a more or less definite curved rise of temperature for three or more readings, and the maximum of the curve is two or more degrees higher than the maximum preinjection temperature.

"Example: Preinjection, (1), 101, (2) 101.4, (3) 101.8. Postinjection, (1) 102, (2) 103, (3) 104.2, (4) 105, (5) 104.1, (6) 103.

"(b) When the temperatures are high and level or nearly equal for 3 to 7 postinjection records. This may be regarded as a reactor.

"Example: Preinjection, (1) 101.4, (2) 101.8, (3) 102. Post 1, (1) 104.4, (2) 104.8, (3) 104.8, (4) 104.4, (5) 104.6. Post 2, (1) 105.1, (2) 105, (3) 105.4, (4) 105.2, (5) 106.

"(c) An early reactor will begin rise in temperature in two to four hours after injection and will not always show a normal or big curve.

"Example: (1) Postinjection, second hour, 104.5; fourth hour, 105; sixth hour, 105; eighth hour, 104; tenth hour, 102. (2) Postinjection, second hour, 104; fourth hour, 104.2; sixth hour, 104.1; eighth hour, 103.4; tenth hour, 101.

"(d) A late reactor may start up at the fifth, sixth, or seventh reading (sixteenth, eighteenth, or twentieth hour postinjection).

"Examples: Post-injection 1, (1) 101.2, (2) 101.4, (3) 101, (4) 102 (5) 101.8, (6) 104, (7) 105, (8) 105.2, (9) 104.8, (10) 103. Postinjection 2, (1) 101.4, (2) 101.6, (3) 102, (4) 101.8, (5) 102, (6) 103.5, (7) 104, (8) 105.1, (9) 104.2, (10) 102.8.

"(e) When there are up and down temperatures, irregular curves, the test may be more or less questionable and the animal is marked suspicious,

¹ Jour. Amer. Vet. Med. Assoc., 1921, lix, 746.

unless cold drinks of water or sudden exercise or excitement may account for the sudden fluctuations of temperatures, and then it is usually best to mark them as suspicious.

"Example: Preinjection, (1) 102, (2) 101.2, (3) 102.2. Postinjection, (1) 102, (2) 104, (3) 101.2, (4) 104.2, (5) 103, (6) 102, (7) 103.

"(f) When the curve is low and does not have a maximum of two degrees above the maximum preinjection temperature, then make the animal a suspect, and give it an ophthalmic test or wait ninety or more days and re-test with intradermal and ophthalmic.

"Example: Preinjection, (1) 101.6, (2) 102, (3) 101.4. Postinjection, (1) 101.5, (2) 101.6, (3) 102.8, (4) 103.2, (5) 103.6, (6) 102.2.

"(g) Always note the physical changes in the reacting animals. Some have a brief chill, the hair stands up and the coat is dry and rough, and some get very thirsty and refuse feed. Some may have a cough, a discharge from the nose, and enlarged lymph glands. Some have tubercles in the udder. The local changes at point of injection must be observed.

"The quantity of standard tuberculin to inject varies with the size, age, and number of the previous injections. If the cattle have never been injected, 4 to 6 c.c. is sufficient for the large cattle and 2 to 4 c.c. for the small and young cattle. There is no doubt about a tuberculous animal becoming tolerant or plugged by frequent small or large doses. A very large dose (10 to 15 c.c.) of tuberculin will often bring forth a reaction in a plugged or tolerant animal, but it is not always certain or positive. As a rule tolerant animals react early and some react exceptionally late. In order to stop owners from plugging animals it is important that all tuberculin production and sales shall be licensed and controlled by Federal and State authorities.

"Temperatures taken before and after injection are very important. The Federal and State regulations specify at least three preinjection and at least six postinjection temperatures two hours apart running to the eighteenth hour postinjection and beginning not later than the eighth hour postinjection. Where every animal is normal and the course is typical this method will catch many or nearly all of the tuberculous animals, but it will not catch the early and late reactors so often found in herds that have been tested several times by the subcutaneous method.

"To my mind we should go back to the old-time original method of taking temperatures. Begin at 4 or 6 A. M. and take preinjection temperatures every two hours until 6 or 10 P. M. Inject at 6 or 10 P. M., and begin at 8 P. M. or 12 midnight and take temperatures every two hours to the twentieth or twenty-eighth hour after injection. When this method is carefully and accurately done, there will be very few mistakes and very few tuberculous animals missed in the test. It may take more time and attention, but it pays in accuracy. The morning preinjection temperatures can be compared with the morning postinjection temperatures. The same is true with afternoon temperatures. Hence the common daily variations give opportunity for a more accurate comparison with same hours each day.

"The good points about the subcutaneous method are:

"1. The numerous data from which to draw conclusions, numerous temperatures, conditions of animals, etc.

"2. In non-plugged animals it is very accurate.

"3. It keeps the veterinarian on his job and gives him a chance to note and see clearly any physical reactions that appear.

"4. It is easily carried on in combination with the ophthalmic method.

"The objectionable points are:

"1. It is more exacting in work and often takes day and night work.

"2. It should cost more on account of more work for the veterinarian and the use of more tuberculin.

"3. It takes about as much time to test one animal as it does for ten.

"4. It is more or less unreliable in plugged animals or animals that have been repeatedly tested by this method.

"5. It cannot be applied to young calves or to wild cattle."

The Conjunctival Tuberculin Test.—For this test Koch's concentrated tuberculin may be used. Preference is usually given to the purified product, prepared as follows: Mix 1 part of Koch's concentrated tuberculin with 20 parts of absolute alcohol. The precipitate that forms is filtered off and dried over sulphuric acid. This powder is then made up into a 4 and 8 per cent. solution in sterile distilled water.

Two or 3 drops of the 4 per cent. dilution are placed in the inner canthus of one eye to sensitize the tissues. After twenty-four hours, unless a positive reaction is present, 2 or 3 drops of the 8 per cent. solution are instilled in the same eye in the same manner. The reaction is usually apparent in from six to twelve hours (Fig. 162).

For the types of reaction and precautions to be observed see the previous description.

One advantage of this test is that the animal will give a reaction in cases where, prior to the test, dishonest dealers have injected tuberculin.

DeFosset¹ has recently described the following method:

"Cases cannot be detected by the ophthalmic method unless the animals are properly prepared for treatment, which should be done by instilling ophthalmic tuberculin in one eye about seventy-two hours before giving the regular test dosage. This preparatory process is known as sensitization. For making the ophthalmic test I would recommend the special disk prepared by the United States Bureau of Animal Industry. There are tablets on the market prepared by the commercial firms which also are satisfactory.

"Technic.—It is most convenient for a right-handed man to treat the left eye of an animal, and we have for this reason chosen the left eye for the test, and for uniformity in work it is necessary that a certain system be followed. An attendant should hold the head of the animal, which is not difficult to do and needs no special restraint, while the operator stands in front of the subject and raises the upper lid of the left eye by grasping it gently with the left forefinger and thumb. The tablet, which is held between the right forefinger and thumb, should be pushed up under the external lateral lid into the conjunctival fornix. After a little experience a deft and skilful operator can do this without injury or pain to the animal. When the tablet has been placed in position the fingers on the left hand should be released from the upper lid and brought down over both lids, holding them together firmly, yet gently, so as not to injure the eye until the tablet has dissolved in the closed conjunctival sac which has been forced by bringing the lids in apposition with each other. Great care must be taken that the tablet is not worked out of the eye before it has had time to dissolve.

"The tablet should not be placed in the inner angle of the eye because the proper tissue structure cannot be treated there, also the membrana nictitans, whose function it is to remove foreign bodies, would remove much of the tuberculin before proper treatment has been effected. The tuberculin should be placed in the upper fornix of the lateral angle because that portion of the conjunctiva is covered with stratified cylindric epithelium containing goblet cells and tubular glands. These cells and glands are capable of secreting mucin when reacting to the toxins in the tuberculin.

¹ Jour. Amer. Vet. Med. Assoc., 1921, lix, 750.

"Reaction.—The criterion of a reaction is a mucopurulent exudate in more or less profusion (Fig. 162). We cannot expect a reaction unless we apply the treatment to tissue capable of reacting. A reaction will usually be preceded by slight redness and lacrimation. A reaction usually does not follow the application of the first tablet, which is employed as the sensitizing agent. The best results follow the application of the second tablet, which should be given about seventy-two hours later. It is not well to hasten the time of sensitization, because the reaction may in some cases be very slight and tend to cause confusion. The best results may be obtained in from three to five days' sensitization. The ophthalmic test should never be concluded by the application of only one treatment. Very careful sensitization is absolutely essential if one would expect satisfactory results. If either the sensitization or the subsequent treatment is carelessly or improperly applied one may note a faint mucous discharge at the inner canthus, which may appear as a little wavy thread, and it is difficult to make an accurate diagnosis and differentiate between the mucopurulent exudate of a tuberculous reaction and a slight mucous discharge commonly seen in normal eyes following the introduction of some foreign substance. A man who would make a successful ophthalmic tuberculin test must be a student of the test and very observing of what takes place after treatment has been placed in the eye.

"Observation should be made if possible in about ten hours after the first tablet has been introduced, and a note should be made of any changes observed and any deviation from the normal or control right eye, using the proper characters for future reference. After the introduction of the subsequent treatment, observations should be made at hourly intervals or often-er, beginning not later than three hours after the instillation of the tablet. Some reactions take place within two hours after the second treatment has been applied. If the treatment has been properly applied, the reactions will be clear and well defined, manifesting themselves first by lacrimation, slight redness of conjunctiva, photophobia, and a free discharge of mucopurulent exudate.

"If there is a tendency of the reaction being somewhat retarded or ill defined, an additional tablet may be given five hours after the second tablet, and this oftentimes tends to give conclusive results if the animal is tuberculous. If there still remains some doubt as to a positive reaction, the animal may be retested within one week by using two tablets without previously sensitizing. Most eyes remain sensitized a week or longer after the subsequent treatment. Very young animals or calves very frequently will give a reaction to one treatment of ophthalmic tuberculin.

"I recommend the tablet form of tuberculin because of the ease with which it can be placed in the proper tissue structures. Only fresh disks which dissolve readily should be employed.

"Recording Reactions.—It is necessary that we adopt the use of some character so that the degree of reaction can be noted as one would note the degree in a thermic reaction. Inasmuch as certain changes take place in the treated eye during reaction, a note for future reference should be made of these changes. These notes should be in such form that they may be recorded on a chart as one would the numerals in thermic reaction. The notations should be made in the order in which the change becomes manifest. For instance, if lacrimation is observed two hours after treatment, the letter L should be placed in that column. If exudation follows next, then the next column should show LX, and so on. The degree of exudation may be noted from X, meaning slight, to XX, well marked, and XXX, very exten-

sive or copious exudation. When only a trace of exudation is observed, it may be noted by writing $\frac{1}{4}$ or $\frac{1}{2}$ X. A positive reaction may be LX or LXXX of varying degree, depending also upon the character of exudation. Hyperemia or redness may be noted by H.

Precautions.—1. Don't treat abnormal eyes or a herd when sore eyes are prevalent.

"2. Don't try to make an ophthalmic test in a barn or corral where the wind is sweeping through and blowing dust, cinders, or other foreign matter.

"3. Don't manipulate the eye of the subject unnecessarily and cause irritation from natural results.

"4. Don't try to put tablets into the eyes of animals before you pare your finger-nails carefully and cleanse the hands.

"5. Don't use irritating chemicals on your hands.

"6. Don't try to see something in the eye that isn't present.

"7. Don't let the owner feel that you have no confidence in your work."

The Cutaneous Tuberculin Test.—In making this test some convenient area is shaved and scraped slightly until serum exudes. A small amount of Koch's old tuberculin is applied to the prepared area. In a positive case a well-marked area of congestion and hyperemia appear at the end of twenty-four hours. This may also be accompanied by a rise in temperature.

The Intracutaneous Tuberculin Test.—In performing this test from 0.2 to 0.4 c.c. of Koch's concentrated tuberculin are injected into the skin through a fine needle. The skin at the root of the tail is generally employed. A white swelling should appear while the injection is being given; if it does not appear, the injection is subcutaneous and unsatisfactory for this test. The appearance of hyperemia and redness with a rise in temperature indicates a positive reaction.

LUETIN REACTION

Stimulated by von Pirquet's discovery of a specific cutaneous reaction for tuberculosis, a number of investigators (Finger and Landsteiner, Wolff-Eisner, Nohe, Ciuffo, Nicolas, Favre, and Gauthier) attempted to obtain a specific reaction for syphilis by applying extracts of syphilitic tissues—prepared from syphilitic fetal liver or chancre—to the skin of syphilitic patients. In spite of some encouraging effects their results were, on the whole, contradictory. Further, Neisser and Bruck found that a reaction similar to that produced with syphilitic extract can be obtained also with a concentrated extract of normal liver. This peculiarity of the skin of syphilitics is ascribed by Neisser to what he calls the state of "*Umstimmung*" in the later stages of syphilis. Both Neisser and von Pirquet expressed the hope and belief that a reaction may be secured by employing an extract of *pallida* free from tissue constituents; this was apparently accomplished by Noguchi,¹ in 1911, first with syphilitic rabbits and then with human patients. Noguchi gave the appropriate name of "*luetin*" to the extract of *pallida*. Theoretically, one should not expect to obtain an allergic reaction in syphilis so long as the activity of *pallida* is maintained at its maximum, or in the very early stages, before there is sufficient time for antibody formation. One can reasonably expect the appearance of the phenomenon when the activity of the microparasite begins to abate through a gradually acquired defensive power of the host, or under an effective therapeutics, as in the later stages of the disease and in hereditary syphilis.

Preparation of Luetin.—At least six different strains of *pallida* in pure culture are being used by Noguchi in the preparation of *luetin*. These are cultivated in ascites-kidney

¹ Jour. Exper. Med., 1911, 14, 557; Jour. Amer. Med. Assoc., lviii, 1163.

agar for periods of six, twelve, twenty-four, and fifty days, at 37° C., under anaërobic conditions. The tubes showing large numbers of spirochetes are then selected, the oil is poured off, the tube is cut, the agar column is removed, and the tissue then cut off. The ascites-agar cultures are then carefully ground in a sterile mortar, the resulting thick paste being gradually diluted with a fluid culture until a homogeneous liquid emulsion is secured. The preparation is next heated for an hour in a water-bath at 60° C., and then tricresol or phenol added to make 0.5 per cent. Cultures are made from this suspension, and rabbits inoculated intratesticularly; both, after suitable intervals, must show an absolutely sterile preparation.

The luetin should be kept in the refrigerator when not in use. The isolation of *Treponema pallidum* in pure culture is a difficult procedure, and, obviously, a luetin must be prepared of pure cultures and from as many different strains as possible. While *pallida* quickly loses its pathogenicity in artificial culture-media and is also highly susceptible to the influence of germicides, its preparation, nevertheless, is an important matter requiring skilful supervision.

A control fluid prepared of sterile agar and bouillon in exactly the same manner as luetin was originally advised by Noguchi, but recently he claims that its use is not necessary.

Method of Application.—Luetin is not applied to an abrasion of the skin, but is injected intracutaneously with a very fine needle and a sterile syringe (Fig. 156). According to original directions the luetin is to be well shaken, diluted with an equal part of sterile salt solution, and 0.07 c.c. injected (0.035 c.c. undiluted). A slightly smaller dose, as, e. g., 0.05 c.c., may be used for children. The skin of the upper arm is usually selected as the site for inoculation. If a control fluid is used, the luetin may be injected into the skin of the left and the control fluid into the skin of the right arm, or both injections may be given in the same arm, about 2 inches apart, the control being above the luetin. As shown by Sherrick,¹ and confirmed by my associates and myself,² normal and non-syphilitic persons taking iodids or bromids at the time the skin test is made or while these drugs are still in the body fluids may yield well-marked non-specific reactions which the physician may readily interpret as a positive luetin reaction.

After cleansing the skin with alcohol it is drawn taut or pinched up between forefinger and thumb and the needle introduced, with the aperture directed toward the outer surface of the skin. If the point of the needle is in the skin, a white elevation occurs immediately upon injecting the solution; if it is in the subcutaneous tissue, no filtration is apparent.

Reactions.—*Normal or Negative Reactions.*—In the majority of normal persons the injection of luetin is followed by a very slight traumatic reaction, or a small erythematous area appears, after twenty-four hours, at and around the point of injection. No pain or itching sensation is experienced; the reaction recedes in forty-eight hours and leaves no induration. In certain individuals the reaction may reach a stage of small papule formation after from twenty-four to forty-eight hours, which subsides within seventy-two hours, leaving no induration.

Positive reactions have been classified by Noguchi into three main varieties:

(a) *Papular Form.*—"A large, raised, reddish, indurated papule, usually 5 to 10 millimeters in diameter, makes its appearance in twenty-four to forty-eight hours. The papule may be surrounded by a diffuse zone of redness and show marked telangiectasis. The dimensions and the degree of induration slowly increase during the following three or four days, after

¹ Jour. Amer. Med. Assoc., 1915, July 31, 404.

² Jour. Amer. Med. Assoc., 1916, lxvii, 718; Jour. Lab. and Clin. Med., 1917, xi, 401.



FIG. 164.—A POSITIVE LUETIN REACTION

E. C., adult male; tertiary syphilis with detachment of the retina; papular lesion of moderate severity; about forty-eight hours after injection of 0.07 c.c. luetin.

which the inflammatory processes begin to recede. The color of the papule gradually becomes dark bluish red. The induration disappears within one week, except in certain instances in which a trace of the reaction may persist for a longer period. The latter effect is usually met with among cases of secondary syphilis under regular mercurial treatment in which there are no manifest lesions at the time of making the skin test. Cases of congenital syphilis also show this reaction (Fig. 164).

“(b) *Pustular Form*.—The beginning and course of this reaction resemble the papular form until about the fourth or fifth day, when the inflammatory processes commence to progress. The surface of the indurated round papule becomes mildly edematous, and multiple miliary vesicles occasionally form. At the same time a beginning central softening of the papule can be seen. Within the next twenty-four hours the papule changes into a vesicle, filled at first with a semi-opaque serum that later becomes definitely purulent. Soon after this the pustule ruptures spontaneously or after slight friction or pressure. The margin of the broken pustule remains indurated, while the defect caused by the escape of the pustular content becomes quickly covered by a crust that falls off within a few days. About this time the induration usually disappears, leaving almost no scar after healing. There is a wide range of variation in the degree of intensity of the reaction described in different cases, as some show rather small pustules, while in others the pustule is much larger. This reaction was found almost constantly in cases of tertiary syphilis, as well as in cases of secondary or hereditary syphilis which had been treated with salvarsan.

“(c) *Torpid Form*.—In rare instances the injection sites fade away almost to invisible points within three or four days, so that they may be passed over as negative reactions. But sometimes these spots suddenly light up again after ten days or even longer and progress to small pustular formation. The course of this pustule is similar to that described for the preceding form.

“This form of reaction has been observed in a case of primary syphilis, in one of hereditary syphilis, and in two cases of secondary syphilis, all being under mercurial treatment.”

Aside from these three types of reactions, there have since been described several cases of the formation of a hemorrhagic exudate, the lesion usually rupturing spontaneously and not running a more severe or a longer course than the pustular. Two such reactions have been reported by Kilgore.¹

“Neither in syphilitics nor in parasyphilitics did a marked constitutional effect follow the intradermic inoculation of luetin. In most positive cases a slight rise in temperature took place, lasting for one day. In three tertiary cases and in one hereditary case, however, general malaise, loss of appetite, and diarrhea were noted.”

In view of the occasional instances in which the reactions are retarded a patient should be observed for two weeks before a reaction is regarded as negative.

Results.—1. The reports of Noguchi and of a number of different observers show that the luetin reaction is generally negative in the primary and secondary (untreated) stages of syphilis.

2. In latent and tertiary syphilis Noguchi has reported positive reactions in from 80 to 95 per cent. respectively, and the reports of others have showed from 64 to 100 per cent. of positive reactions.

3. In cerebrospinal syphilis positive reactions have been reported in from 42 to 80 per cent. of cases.

¹ Jour. Amer. Med. Assoc., lxii, 1236.

4. In congenital syphilis the results have varied within wide limits—10 to 96 per cent. of positive reactions. In cases under one year of age Noguchi has reported about 23 per cent., and among later cases 96 per cent., of positive reactions.

5. Second injections of luetin apparently do not give positive reactions in non-syphilitic cases.

Non-specific Reactions and Practical Value.—A large literature has accumulated on the use of the luetin reaction as a means for the diagnosis of syphilis, but not a few investigators have reported non-specific or doubtful reactions with the comment that control fluids of the culture-medium alone yielded reactions of the same or almost the same kind and degree.

The most thorough investigation bearing upon the non-specific phase of the luetin reaction has been that of Stokes.¹ This investigator has observed that the intracutaneous injection of 0.5 per cent. solutions of sterile agar and emulsions of normal skin elicit reactions in syphilis quite similar to those produced by luetin. Stokes has explained these reactions on the basis that agar and other substances are capable of absorbing antiferments and thereby liberate or render active proteolytic ferments capable of producing toxic or irritating substances by processes of proteolysis and autolysis of the individual's serum and cells responsible for the inflammatory reactions of papulation and pustulation. Stokes has very clearly described this mechanism in the following words: "While the luetin reaction seems several steps removed from the plainer case of the agar reaction, a close examination of experimental evidence bearing on it gives ground for placing it, in large part at least, in the class of antiferment-absorbent or anaphylatoxin reactions, and treating it as in the case of the reaction to agar, not as specific for syphilis, but as a measure, albeit, perhaps a sensitive one, of the ferment-antiferment balance, and of the amount or intensity of action of non-specific proteases in the body of the syphilitic."

Stokes believes that with luetin the processes are of a dual nature, that is, that antiferment is absorbed not only by the ascites agar, but likewise by the fragments of *Spirocheta pallida* in which case the injection of luetin may be expected to yield somewhat stronger reactions than the control fluid of culture-medium (ascites fluid and agar) alone, but that both processes are essentially non-specific.

In my opinion studies of this kind interpreted in conjunction with the fundamental observations of Jobling, Eggstein, and Petersen upon ferments, antiferments, and their balance, offer satisfactory explanations for the production of reactions following the injection of various substances (agar, blood, kaolin, gelatin, tissue, and bacterial extracts, etc.) into the skins of healthy and diseased individuals; they do not, however, explain specific allergic skin reactions and notably the intracutaneous tuberculin reaction, which may be elicited by the injection of 0.1 c.c. of a 1 : 10,000 dilution of O. T. carrying an almost infinitesimal amount of glycerin-bouillon incapable of eliciting these non-specific reactions. In other words, the physical theory of anaphylatoxin production enlisted for explaining the agar reaction is not acceptable in explanation of specific allergic reactions which is very probably an intracellular colloidal phenomenon without the essential production of such toxic substances as "anaphylatoxins," "proteotoxins," and the like.

Very probably the intracutaneous injection of luetin may elicit a dual reaction, one non-specific due to the absorption of antiferment by the agar, ascites fluid, and even fragments of *Spirocheta pallida*, and the other specific, a true allergic reaction to pallida protein. But the latter has not been proved

¹ Jour. Infect. Dis., 1916, 18, 402, 415, Jour. Amer. Med. Assoc., 1915, 65, 404.

and the experiments of Greenbaum and the writer¹ have questioned whether true cutaneous allergy to culture pallida actually exists. On the other hand, it cannot be questioned that the intracutaneous injection of luetin into syphilitics has usually elicited more frequent and stronger reactions than observed among non-syphilitics; this difference may be ascribable, however, more to the development of non-specific and non-allergic hypersensitiveness of the skin in syphilis, an enhancement of the non-specific ferment-antiferment activities, than to the development of specific allergic sensitiveness to the proteins of *Spirocheta pallida*. It was for the purpose of shedding more light if possible upon the question of whether or not true cutaneous allergy developed in syphilis that our experiments were conducted.

In our investigations three products were employed:

(a) Ascites agar luetin prepared according to the original method of Noguchi of six or more strains of pallida originally isolated by Noguchi and, therefore, many years removed from syphilitic lesions.

(b) Ascites agar culture-medium in exactly the same proportions as contained in the luetin and prepared in exactly the same manner.

(c) A saline suspension of washed *Spirocheta pallida*. Naturally most interest centered in these preparations as being most likely to yield true information on the occurrence of intracutaneous reactions to the fragments and products of pallida alone and free of the constituents of culture-media.

These vaccines of *Spirocheta pallida* were prepared by cultivating the same strains as used for preparing the regular luetin in fluid media and removing them by thorough centrifuging. The sediment of spirochetes was then washed at least once with sterile saline solution and resuspended in sufficient saline solution to give a turbid suspension showing twelve or more spirochetes in each dark field by microscopic examination. The number of spirochetes in each unit volume was, therefore, somewhat higher than present in ordinary luetin.

These suspensions were thoroughly shaken with beads, heated at 60° C. for one hour, cultured for sterility, and preserved with 0.3 per cent. tricresol.

Each of the three products were injected intracutaneously into groups of syphilitic and non-syphilitic individuals, the skin of the forearms being employed.

The amount injected was always 0.1 c.c. of each, but varying dilutions of the stock products were injected in order to elicit possible differences in the reactions. For example, the regular ascites agar luetin was injected in dose of 0.1 c.c. undiluted, 1 : 2, 1 : 4, 1 : 8, 1 : 16, etc., at the same sitting. The control fluid of ascites agar was injected at the same time in the other arm in similar amounts. The pure luetin or vaccine of washed spirochetes was injected in dose of 0.1 c.c. undiluted, 1 : 2 and 1 : 4, higher dilutions not being employed because of the complete absence of all reaction except that ascribable to trauma.

By injecting the luetin and its control in serial dilutions and then measuring the reactions at the end of forty-eight hours it was hoped to elicit differences ascribable to the presence of pallida and that these may be checked up by the reactions engendered by pallida alone in the washed suspension.

Without detailing or tabulating the results of a large number of tests, it may be stated that the reactions engendered by the regular luetins and their controls were almost identical in degree and extent. Minor differences occurred in some instances as might be expected, but these were not constant, in that sometimes a given dilution of the control fluid gave a slightly stronger reaction than the corresponding dilution of the luetin and the reverse.

¹ Jour. Amer. Med. Assoc., 1922, 79, 2063.

The intracutaneous injection of the pallida vaccine in dose of 0.1 c.c. sometimes produced well-defined areas of erythema and induration in syphilitics, but milder reactions of the same kind were observed among non-syphilitics, and in any set of tests conducted on the same day with both classes of patients reactions of similar kind and degree were observed in both. In general terms the syphilitic patients usually showed somewhat larger and better defined reactions which we ascribed to the increased non-specific ferment-antiferment cutaneous processes occurring in this disease.

Our results indicate one of two possibilities: (a) either true cutaneous allergy to pallida protein and products does not occur in syphilis and that the ordinary luetin reaction is purely a non-specific physical reaction or, (b) some change has occurred in the culture pallida employed in our work and for the manufacture of luetin in general, reducing or removing their allergenic or anaphylactogenic activities.

That the latter is no idle possibility is indicated by the recent report of Alderson,¹ who has compared the results obtained with luetin made at present by different commercial firms with those observed some years ago after luetin was first prepared by Noguchi. Owing to the frequency of negative reactions among syphilitic subjects Alderson has concluded that the luetin available at present may be inert and wisely cautions against its use for diagnostic purposes.

In view of the fact that allergic reactions in tuberculosis and some other bacterial and mycotic infections may be elicited by preparations of organisms long in artificial cultivation it would appear improbable that *Spirocheta pallida* could lose to such a large extent in allergenic qualities; future investigations conducted with freshly isolated pallida must be awaited to settle the question, but with the culture pallida not available our results indicate that either true and specific cutaneous allergy to pallida does not develop in syphilis at all or but to a slight degree, or that the culture pallida have actually lost in allergenic activity, as indicated by the work of Alderson.

MALLEIN REACTION

Mallein is a glycerin extract containing the toxic principles of the *Bacillus mallei*, the microorganism causing glanders. It is used entirely as a diagnostic agent in veterinary practice, but may also be used for the diagnosis of human glanders, the dosage being the same as that of old tuberculin.

Two methods are commonly employed: (1) The subcutaneous injection and (2) instillation into the eye (ophthalmic test).

Method of Preparing Mallein.—A pure culture of *Bacillus mallei* is usually obtained by injecting a male guinea-pig with infected material, and at the end of twenty-four or forty-eight hours isolating a pure culture from the testicle.

The micro-organism is grown for from six to eight weeks in special bouillon containing 5 per cent. glycerin, at 37° C., similar to tuberculin. Unlike the tubercle bacillus, glanders bacillus grows evenly through the bouillon instead of upon the surface.

At the end of six or eight weeks the flasks are removed from the incubator and placed in a sterilizer at 100° C. for at least two hours. This process kills the bacilli and extracts the toxic principles. The entire solution is evaporated down to one-tenth of its volume, filtered in small bottles, and sterilized. This is called concentrated mallein, and is kept in this form until ready for use.

Before using it is diluted to its original volume with 0.5 per cent. phenol solution, and passed through a porcelain filter. This process removes all the bacilli, and renders the solution aseptic and ready for use.

Subcutaneous Mallein Reaction.—The dose of mallein for a horse is 0.4 c.c. of concentrated mallein, or 4 c.c. or 1 dram of the diluted product.

¹ Arch. Dermat. and Syph., 1922, 5, 610.

The dose for a retest is 0.8 c.c. of concentrated or 8 c.c. or 2 drams of the diluted solution. Mallein is injected subcutaneously in some convenient area, such as around the shoulder, which has been shaved and cleaned.

A positive reaction is based on the same principle as tuberculin, that is, a rise of temperature within twenty-four hours following the injection, with a local inflammatory reaction at the site of injection.

Ophthalmic Mallein Reaction.—*Ophthalmic mallein desiccated*, first employed by Meyer,¹ is prepared by taking 1 part of concentrated mallein and adding 20 parts of absolute alcohol. This forms a precipitate that is filtered and dried in a desiccator over sulphuric acid. A 5 per cent. solution of the powder is made with sterile water. Two or 3 drops of the aqueous solution are dropped into the inner canthus of one eye. In case of a positive reaction this is followed by a marked conjunctivitis, associated with a purulent exudate extending from the inner canthus similar to the reaction shown in Fig. 162. In most cases there is also a rise of temperature. Only one dose is to be applied in the ophthalmic mallein test. It is not considered necessary to sensitize the eye, as in tuberculosis. The ophthalmic mallein test has the same advantages as the ophthalmic tuberculin test, that is, one can obtain a reaction when dishonest horse dealers have injected mallein prior to a subcutaneous mallein test, also in cases of far-advanced glanders, which at times give no reaction to a subcutaneous injection of mallein.

Ferry² has prepared a convenient tablet of dried mallein for the ophthalmic test similar to the tablet of tuberculin previously described. Each tablet contains the exact amount of mallein required for the test. Instead of dissolving the tablet in water prior to its application, as has previously been done with desiccated mallein, the tablet is placed directly into the conjunctival sac at the inner canthus of the eye and there allowed to remain. The tablet will soon (one to three minutes) dissolve without apparent discomfort or annoyance to the animal and without an irritating effect upon the conjunctiva. The mallein which is thus set free produces typical reactions similar to those recorded as the result of the instillation of the raw mallein, or the solution of the dried mallein.

The ophthalmic mallein test is preferred to the subcutaneous and has been especially endorsed by the American Veterinary Association.³ The French veterinarians appear to prefer an intrapalpebral test, but this requires the use of a syringe and is more laborious. Mason⁴ and others have found the complement-fixation test of greatest value, the agglutination test second, and the mallein test third, for the diagnosis of glanders in horses and mules.

ABORTIN REACTION

Bang⁵ has observed that artificially infected animals reacted with a marked rise of temperature, loss of appetite, and slight diarrhea when injected subcutaneously with a culture of *Bacillus abortus*, which he had established as the course of infectious abortion of cattle. The English Commission⁶ prepared and used a glycerin extract of *Bacillus abortus* in the same manner as tuberculin, and with success; Meyer and Hardenbergh⁷ have prepared a precipitated purified abortin with which highly specific

¹ Jour. Infect. Dis., 1913, 12, 170.

² Jour. Amer. Vet. Med. Assoc., October, 1916.

³ Amer. Vet. Rev., 1913-14, 44, 218.

⁴ Jour. Immunology, 1920, 5, 489.

⁵ Ztschr. f. Tier., 1897, 1, 241; Archiv. f. Wissen. und Prakt. Tierhl., 1907, 33, 312.

⁶ Report of Departmental Committee on Epizootic Abortion, London, 1909, Part I.

⁷ Jour. Infect. Dis., 1913, 13, 35.

reactions have been observed; Reichel and Harkins¹ have employed an intradermal test with a suspension of heat-killed and washed bacilli. The results of these investigations indicate that the abortin test is highly specific; as with other anaphylactic reactions, this test does not serve to differentiate the actively infected from the recovered animals, but when applied to a herd will show whether or not Bang's disease is or has been a source of infection among the animals.

According to Fleischner and Meyer the test has been uniformly negative in infants, indicating that *Bacillus abortus equinus* is probably not pathogenic for human beings and that infection does not occur through the ingestion of milk.

ALLERGIC REACTIONS IN TYPHOID FEVER

Historic.—In 1907 Chantemesse² observed characteristic inflammatory symptoms follow the installation of typhoid bacilli extract into the eye of patients suffering from typhoid fever. Kraus³ and his associates, repeating these experiments, could not convince themselves of the specificity of this reaction, stating that healthy individuals also give it to some extent, and that other bacterial extracts cause similar symptoms in typhoid-fever patients. In addition, he tried a cutaneous reaction, but without result. Zupnik,⁴ on the contrary, states that a cutaneous reaction is useful, while the ophthalmic reaction is not useful. Deehan⁵ obtained a weak to moderate reaction in 12 cases of typhoid fever, whereas 8 control cases showed none. Floyd and Barker⁶ obtained positive results in 19 out of 20 cases and none in 18 controls, including 2 cases of paratyphoid fever. Chaufford and Trosier⁷ reported unfavorably on the reaction.

Austrian⁸ has reported very favorably upon an *ophthalmic reaction in typhoid fever* following the installation of "typho-protein" prepared by cultivating a large number of different strains of typhoid bacilli, precipitating the protein with alcohol, drying the precipitate, and redissolving in water so that from $\frac{1}{3}$ to $\frac{1}{2}$ milligram is contained in each drop. In typhoid-fever patients reaction of the palpebral conjunctiva of the lower lid and of the caruncle appears on an average of two and a half hours later, reaching the maximum about the sixth hour, and usually subsiding within forty-eight hours. In 75 cases of typhoid fever this test was found positive in 71 and negative in 4. In 3 cases the eye test antedated the Widal reaction, and in only 23 per cent. was the Widal reaction positive at as early a date as the eye test. A study of 190 persons normal or ill with diseases other than typhoid has convinced Austrian of the specificity of the test, and he recommends it as an aid to diagnosis on account of its simplicity and the absence of any discomfort to the patient.

Typhoidin Test of Gay and Force.—Gay and Force⁹ have reported favorably upon a *cutaneous reaction* indicative of immunity against typhoid fever. The preparation which they used, "typhoidin," is prepared in the same manner as Koch's old tuberculin: 250 c.c. of a 5 per cent. glycerin bouillon is inoculated with *Bacillus typhosus* and cultivated at 37° C. for

¹ Jour. Amer. Vet. Med. Assoc., March, 1917.

² Deut. med. Wchn., 1907, 33, 1572.

³ Wien. klin. Wchn., 1907, 20, 1335.

⁴ Münch. med. Wchn., 1908, 45, 148.

⁵ Univ. Penn. Med. Bull., 1909, 22, 6.

⁶ Amer. Jour. Med. Sci., 1909, 38, 188.

⁷ Compt. rend. Soc. de biol., 1909, lxvi, 519.

⁸ Bull. Johns Hopkins Hosp., 1912, 23, 1.

⁹ Archiv. Int. Med., 1914, 13, 471.

five days. It is then reduced, without filtration, to one-tenth of its original volume by evaporation over an acetone bath for about eight hours. A control solution of sterile 5 per cent. glycerin bouillon is prepared in the same manner.

The skin of the forearm is cleansed with alcohol, and two abrasions are made with the von Pirquet borer, as described under the cutaneous tuberculin test. The "typhoidin" is applied to one cut and the control fluid to the other. The reactions are observed six and twenty-four hours later. Occasionally there is a traumatic reaction in the control, but a positive reaction may be detected by a wider areola and increased induration.

Positive reactions were secured in 95 per cent. of cases that had recovered from typhoid fever, 2 of the cases having had the disease respectively forty-one and thirty-three years before. The reaction was found negative in 85 per cent. of individuals not having typhoid fever. Of 15 persons immunized by the army method, from four and three-quarter years to eight months previously, nine gave a positive skin reaction. Twenty-four individuals immunized by a sensitized vaccine (Gay and Claypoole) for from one to eight months previously reacted positively. Later Gay and Claypoole¹ prepared typhoidin by precipitating the solution with alcohol, washing the precipitate with alcohol and ether, drying in a vacuum, and suspending the resulting powder in phenolized normal salt solution which was injected intracutaneously and applied cutaneously; a control powder was prepared from broth and used in the same manner. With this skin test Gay and his associates have studied the relative value of various vaccines and regard the anaphylactic reaction as indicative of a state of immunity. Nichols² has questioned the value of the anaphylactic skin test as an index of immunity and regards the reaction as indicating nothing more than sensitization to typhoid protein, which is apparently less lasting and less specific than the true immunity to this infection. He bases this opinion on the fact that in his experience the typhoidin skin test gave fewer positive reactions (75 per cent.) than generally expected, as about 90 per cent. of persons who have had typhoid fever are immune for many years or even for the balance of life. Furthermore, according to Nichols, experience has shown that protection following typhoid fever is of longer duration than is indicated by the typhoid in test, and while a large percentage of persons who have had typhoid fever or have been immunized with typhoid vaccine react to paratyphoidin, recent experiences and statistics, particularly in Europe, have indicated that these persons are not immune to paratyphoid fever. Kilgore³ has reported favorably upon the value of the typhoidin cutaneous test; Austrian and Bloomfield⁴ found that the test failed to furnish data by means of which it was possible to differentiate between those who had neither typhoid fever nor had received the vaccine and those who had either had the disease or had been immunized.

In our own experience⁵ powdered typhoidin and its control produced severe reactions when injected intracutaneously in doses of 0.0005 to 0.001 mgm.; these reactions and particularly that produced by the control rendered the reading and interpretation of the test quite difficult and subject to much error.

Force and Stevens⁶ later prepared a stable preparation of typhoidin which may be rapidly prepared by precipitating a concentrated broth culture of *Bacillus typhosus* with 95 per cent. alcohol, and subsequent

¹ *Archiv. Int. Med.*, 1914, 14, 671.

² *Jour. Exper. Med.*, 1915, 22, 780.

³ *Archiv. Int. Med.*, 1916, 17, 25.

⁴ *Archiv. Int. Med.*, 1916, 17, 663.

⁵ *Jour. Immunology*, 1916, 1, 409.

⁶ *Archiv. Int. Med.*, 1917, 19, 440.

dehydration with absolute alcohol and absolute ether. According to these investigators it is imperative that no account be taken of the appearance of the reaction at the end of twenty-four hours. A positive typhoidin reaction is indicated by the presence, forty-eight hours after the test, of a well-defined erythematous papule at least 5 mm. in diameter.

Out of 18 normal persons, 17 gave negative reactions; out of 25 persons with a history of typhoid, 19 gave positive reactions, 1 gave a doubtful, and 6 persons (4 with questionable typhoid histories) gave negative reactions. Out of 152 persons previously vaccinated against typhoid, 12 of 56 vaccinated during 1916, 3 of 11 vaccinated during 1915, 13 of 29 vaccinated during 1914, and 26 of 52 vaccinated during 1913 gave negative reactions.

Meyer and Christiansen have likewise emphasized the importance of the method employed for the preparation of typhoidin in order to reduce the degree of non-specific irritation.

Cutaneous anaphylaxis to typhoidin was found apparently to persist for a longer time among those who have had typhoid fever than among those actively immunized with the vaccine. Among the latter the highest percentage of reactions was found during the first year following immunization. The test was advocated as a means of determining whether or not a person possesses immunity to typhoid fever either acquired by recovery from the disease or by artificial immunization; the sum total of researches by various investigators is to the effect that this skin test is an indication of hypersensitiveness to typhoid protein, but cannot be accepted at present as an indication of immunity.

Berge and myself¹ found no relation between cutaneous allergy and the presence of antibodies, and Meyer and Christiansen² observed exactly similar results in experiments with rabbits. The latter observed that a positive typhoidin skin reaction in a rabbit does not indicate that this animal will resist a subsequent intravenous injection of living typhoid bacilli, or that the animal is so protected that it will not become a chronic carrier of bacilli in gall-bladder or liver. And again, a marked hypersensitiveness of a typhoid-immune rabbit to fowl typhoidin does not indicate the presence of a resistance to an infection with the same organisms.

Force and Stevens,³ however, have maintained that the original assumption of Gay and Force is true that the typhoidin test may be used as a measure of protection against typhoid. They state that even granting that typhoid immunity is of longer duration than cutaneous sensitiveness to typhoid protein, the disappearance of this sensitiveness furnishes an indication for revaccination of the person, and still allows a margin of safety within the as yet indefinite limits of typhoid immunity. There is as yet no evidence that a positive typhoidin test is not indicative of protection against typhoid fever. In two instances at least, in their experience, a negative typhoidin test after vaccination was followed by typhoid infection.

ALLERGIC REACTIONS IN OTHER DISEASES

Gonococcus Infections.—In 1908 Irons⁴ reported general and local reactions in persons suffering from gonococcus infections following the subcutaneous injection of gonococcal vaccines. This reaction has been observed by Bruck⁵ in epididymitis, by Reiter⁶ in pelvic infections in women, and also by other observers in other conditions.

¹ Jour. Immunology, 1916, 1, 409.

⁴ Jour. Infect. Dis., 1908, v, 279.

² Jour. Infect. Dis., 1917, 20, 357-441.

⁵ Deut. med. Wchn., 1909, xxxv, 470.

³ Archiv. Int. Med., 1917, 19, 440.

⁶ Ztschr. f. Geburtsh. u. Kinderh., 1911, lxxviii, 471.

Experiments with glycerin extracts of the gonococcus prepared from several strains, singly or combined in one preparation, have yielded Irons,¹ well-defined cutaneous reactions. These tests were conducted after the method of von Pirquet's tuberculin test.

Irons found that the cutaneous inoculation of glycerin extracts of autolyzed gonococci in patients infected by the gonococcus produces a well-defined reaction. This reaction is not usually obtained in normal persons, nor in those suffering from other infectious diseases. In persons recently infected, the reaction is negative and increases gradually during the course of the disease. In the more chronic forms of gonococcal infection, such as arthritis, the degree of the cutaneous reactivity varies from day to day, and these variations may be correlated with the changes in the clinical course of the disease. Cases of severe infection, such as extensive arthritis, may give negative reactions. Later, when improvement has occurred, the reaction becomes positive. In general, a positive reaction is obtained in patients with gonococcal infection at some time during the course of the disease. In normal persons the *gonococcin* prepared in the manner described gives a cutaneous reaction rarely more than 2 to 3 mm. in diameter. Occasionally in adults and somewhat more frequently in children fairly marked reactions are met with where previous gonococcal infection can be excluded. In these cases the normal antibodies may be increased to an unusual degree. The cutaneous reactions obtained with meningococcal and gonococcal antigens suggest that we are dealing with a group reaction. In diagnosis, a positive reaction is to be regarded as confirmatory evidence of gonococcal infection. Other infections, such as those by the meningococcus or *Micrococcus catarrhalis*, which may give rise to a group reaction, must be excluded. The clinical value of the reaction must be determined by further tests and its limitations defined by a study of many groups of cases.

Diphtheria.—Schick has advocated the intracutaneous injection of a minute dose of diphtheria toxin as a test for antitoxin in the serum of an individual. If sufficient antitoxin is present, the toxin is neutralized and no local disturbances are apparent; otherwise local inflammatory areas may be observed. This test is not regarded as an allergic reaction; a further description of it will be found in Chapter XXXII.

Recently Dr. Moshage and myself² have applied an anaphylactic skin test in diphtheria with a polyvalent antigen designated *diphtherin*; positive reactions were observed in about 70 per cent. of children and 35 per cent. of adults, and the test was of practical interest mainly from the viewpoint that the anaphylactic reaction may be mistaken for a positive Schick reaction.

Pneumonia.—Many attempts have been made to devise a skin test for pneumococcus pneumonia not only for possible differential diagnosis of types of pneumococci, but more especially as studies upon immunity in this disease and the mechanism of the crisis.

Clough³ employed cutaneous and ophthalmic tests with pneumococcus protein secured by precipitating autolyzed pneumococci with alcohol, with practically negative results.

Weil,⁴ employing suspensions of Type I and Type III pneumococci in water heated to 60° C. injected intracutaneously, observed no reactions during the course of the disease, but after crisis a considerable percentage yielded

¹ Jour. Amer. Med. Assoc., 1912, lviii, 931; Jour. Infect. Dis., 1912, 11, 77.

² Amer. Jour. Dis. Children, 1916, xii, 316.

³ Johns Hopkins Hosp. Bull., 1913, 24, 295.

⁴ Jour. Exper. Med., 1916, 23, 11.

positive reactions; positive reactions were also observed in some controls which Weil believed may have been due to unrecognized sensitizations to pneumococcus proteins.

Steinfeld and myself¹ prepared antigens of pneumococci of Types I, II, and III by suspending the cocci in saline solution and heating to 60° C.; the amount injected intracutaneously was 0.1 c.c. or 200,000,000 cocci. Of 19 cases of lobar pneumonia, 6 gave positive reactions with one or more antigens. But in none did the type or types of pneumococci found in the sputum exactly correspond with those shown by the skin reaction, and we concluded that the allergic reactions to pneumococcus protein are of a more general character than the agglutination reactions. The earliest reaction occurred three days after the crisis and a large number of controls reacted negatively.

Weiss and myself² have also prepared "pneumotoxin" by autolyzing young cultures of virulent Type I pneumococci in 2 per cent. solution of sodium oleate. This material represented a thermolabile toxin which had to be freshly prepared. Tests were conducted by injecting 0.1 c.c. intracutaneously, which represented one-twentieth the minimal lethal dose for guinea-pigs. A control fluid was prepared in the same manner.

This toxin was analogous to the diphtheria toxin employed in the Schick test for immunity to diphtheria. Positive reactions were observed in 38 cases of lobar pneumonia as early as the fifth day (the earliest under observation) and as late as the thirteenth. Positive reactions were also observed in 5 of 16 cases after the crisis and convalescent; 23 controls yielded negative reactions.

While we considered these positive reactions due to sensitization to pneumotoxin, it is possible that the reactions were not altogether allergic, but the result of direct irritation of the skin just as diphtheria toxin is an irritant unless neutralized by antitoxin. Positive reactions may indicate the absence of neutralizing principles early in pneumonia, but these may be developed later in the disease and account for the high percentage of negative reactions after the crisis.

Bigelow³ has studied the subject of allergic skin reactions in pneumonia employing ten different kinds of antigen of all four types of pneumococci. Of 104 cases of lobar pneumonia tested, 11 (10.5 per cent.) gave specific type reactions, 46 (42.3 per cent.) gave a common reaction, and 52 (50 per cent.) gave no such reaction. Of the 20 controls, none gave a specific type reaction, 9 gave a common reaction, and 11 gave no reaction. Best results were observed with an antigen prepared by autolyzing pneumococci in saline solution or distilled water.

While apparently specific type intracutaneous reactions may be observed in pneumonia, these do not occur early enough to be of service in directing serum therapy; a second non-specific reaction, differing in time and character from the specific type reactions, may occur in cases of pneumonia and controls.

Meningococcus Carriers.—Gay and Minaker⁴ have prepared *meningococcin* from six strains of normal and parameningococci by precipitating saline suspensions with alcohol, washing the sediment with ether, and drying. This material was ground into a fine powder and suspended in physiologic saline solution containing 0.5 per cent. phenol in such proportions that

¹ Jour. Infect. Dis., 1917, 20, 344.

² Jour. Immunology, 1918, 3, 395; Arch. Int. Med., 1923, 31, 263.

³ Archiv. Int. Med., 1922, 29, 221.

⁴ Jour. Amer. Med. Assoc., 1918, 70, 215.

0.05 c.c., the amount injected intracutaneously, contained 1/150 mg. of the powder.

A reaction was interpreted as positive when presenting at the end of twenty-four hours an areola of 3 to 7 mm. with distinct induration. Of 31 positive carriers, the test was positive in 20, or 64.5 per cent.; of 38 carriers, the test was positive in 10, or 26.4 per cent.

The authors have drawn no conclusions as to the significance of these reactions, but suggest that this intradermal test may prove of aid in the detection of those carrying meningococci for sufficient time to effect sensitization, and that positive reactions may indicate the acquisition of some degree of resistance.

Pertussis.—Modigliani and Villa¹ have recently described an intradermal test for whooping-cough. A loop of a culture of the Bordet-Gengou bacillus in 1 c.c. of distilled water containing a little of a 3 per cent. solution of toluene, was injected into the skin by the tuberculin intradermal technic. The dose for each child was 0.1 c.c. No response was obtained with various diseases other than whooping-cough, while an inflammatory reaction formed constantly in the 38 children with pertussis. The reaction was negative also in 10 children that had recovered from whooping-cough. The results were most instructive in 3 children who had been exposed to pertussis, but showed no signs of it at the time. They gave a pronounced positive response to the injection, and a few days later the symptoms of pertussis developed.

Orgel² has recently applied an intracutaneous test by injecting 2 minims of a vaccine of *Bacillus pertussis* containing 2,000,000,000 per cubic centimeter. He states that specific reactions were observed that sensitization occurs early enough in the disease to offer hope that the skin test may prove of value in early diagnosis, at least before the paroxysmal stage.

Sporotrichosis, Blastomycosis, and Coccidiosis.—Meyer³ and Moore and Davis⁴ have reported positive reactions conducted by the intracutaneous injection of *sporotrichin* and may prove of practical value in the diagnosis of sporotrichosis.

Stober⁵ has reported negative skin and ophthalmic reactions in blastomycosis.

Cooke⁶ has reported negative cutaneous and intracutaneous reactions in coccidiodal granuloma.

Smallpox; Cowpox; Chickenpox.—As previously stated, Jenner in 1798 first described skin reactions when reporting his failure to inoculate 14 persons with cowpox who had previously had smallpox:

"It is remarkable," writes Jenner, "that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces smallpox. Indeed, it becomes almost a criterion by which we can determine whether the infection will be received or not. It seems as if a change which endures through life has been produced in the action or disposition to action in the vessels of the skin; and it is remarkable, too, that whether this change has been effected by smallpox or the cowpox that the *disposition to sudden cuticular inflammation* is the same on the application of variolous matter."

This "sudden cuticular inflammation" of Jenner is recognized by von Pirquet as "the immediate reaction" which follows attempts to vaccinate

¹ *Pediatrica*, Naples, 1921, 29, 337.

² *Jour. Amer. Med. Assoc.*, 1922, 79, 1508.

³ *Jour. Amer. Med. Assoc.*, 1915, 65, 579.

⁴ *Jour. Infect. Dis.*, 1918, 23, 252.

⁵ *Arch. Int. Med.*, 1914, 13, 509.

⁶ *Arch. Int. Med.*, 1915, 15, 479.

those how have recovered from smallpox or have been recently vaccinated. It indicates, in other words, that protection already exists in that individual, that antibodies are present which destroy the vaccine colony and prevent the evolution of the vaccine pustule. Force has suggested that *this immediate or "immunity reaction"* (see Fig. 173) *should be looked for in twenty-four hours*, as its presence is indicative of immunity to cowpox and smallpox and would obviate the repeated and vain attempts to produce true vaccinia in such an already protected person.

Force and Beckwith¹ have further employed a localized reaction of this type in the differential diagnosis of smallpox. They find that rabbits may be immunized against vaccinia or variola by subcutaneous injections of vaccine virus. Such animals show a specific local reaction on the intradermal injection of vaccine virus or of pus from a smallpox vesicle. They do not, however, react to varicella material. Force² has recently been able to obtain an early diagnosis in 2 cases of suspected smallpox by this method. One drawback to the method, however, is the difficulty of giving rabbits intradermal injections on account of their thin skins; sometimes these injections are facilitated by using the skin of the ears instead of the abdomen or back.

Tieche³ has employed similar tests for the differential diagnosis between smallpox and chickenpox by inoculating himself and 2 other volunteers immune to smallpox with the material from the lesions of the patient. The lymph from the patient is first heated to 60–70° C. for five minutes and then applied to scratches on the arm; if the patient has smallpox a reaction occurs in a few hours, whereas in the case of chickenpox no local reaction is evident for several days.

Echinococcus Disease.—In echinococcus disease of the liver and spleen Pontano⁴ and Luridiana⁵ have employed intradermal tests for diagnosis consisting of the injection of 0.2 to 0.3 c.c. of cyst fluid. They quote Casoni as having previously used this test successfully for diagnosis employing the fluid from cysts of the lower animals preserved with phenol. Pontano reports positive reactions in 84 per cent. of cases, suppuration of the cysts being responsible for the negative reactions sometimes obtained. Luridiana reports 5 positive reactions in 6 patients, the negative reaction occurring in a patient with cyst walls unusually fibrous, which probably interfered with sensitization. Serra⁶ reports positive skin reaction in 90 per cent. of cases of echinococcus disease of the liver and spleen. Negative reactions were ascribed to thickened cyst walls limiting sensitization and to processes of desensitization. Complement-fixation and skin reactions are believed capable of correctly diagnosing the majority of cases.

Ringworm and Favus.—Bloch and Massini⁷ have reported positive skin reactions to *trichophyton* among animals immunized with ringworm cultures. Amberg⁸ studied the test in a large group of individuals by applying trichophyton to abrasions of the skin and reports a high percentage of positive reactions. Strickler and myself have prepared antigens of various cultures of ring-worm and favus by grinding the mycelia with sodium chlorid and suspending in sufficient water to render the solutions isotonic, followed

¹ Jour. Amer. Med. Assoc., 1915, 65, 588.

² Jour. Amer. Med. Assoc., 1916, 66, 1384.

³ Corresp. Blatt. f. Schw. Aertze, 1913, 44, 1121.

⁴ Policlinico, 1920, 27, 1921, 28, 41, 405.

⁵ Policlinico, 1921, 28, 41, 399.

⁶ Policlinico, 1921, 28, 35.

⁷ Ztschr. f. Hyg., 1909, lxiii, 68.

⁸ Jour. Exper. Med., 1910, 12, 435.

by sterilization with heat and preservation with phenol. The intracutaneous injection of 0.05 to 0.1 c.c. of this *trichophytin* into individuals with body ringworm was followed by positive reactions, whereas with *favin*, negative or weakly positive reactions occurred. With individuals with favus the intracutaneous injection of *favin* was followed by positive reactions, whereas the *trichophytin* yielded negative or much weaker reactions.

Pregnancy.—From time to time the possible allergic nature and mechanism of labor is discussed in medical literature. Heide¹ in 1911 advanced experiments in support of this theory, but in my own experiments² I was unable to demonstrate any anaphylactic influence upon guinea-pigs at or near term by the intravenous injection of maternal pig sera collected just before or just after labor; nor by the injection of sera of newborn pigs and placental extracts. Thies³ believed that eclampsia may be an allergic phenomenon, but Johnstone⁴ secured no support for this hypothesis in his experiments.

Engelhorn and Wintz⁵ and Esch⁶ claim to have observed positive skin reactions among pregnant women with preparations of placenta designated as *placentins*. Williams and myself⁷ also observed reactions of this character among pregnant women and those who have borne children, but neither we nor Falls and Bartlett⁸ found them constant or of diagnostic value; they occur, however, in late pregnancy and are significant from the standpoint of possible sensitization with placental protein.

Cancer.—Analogous to these attempts toward demonstrating allergic skin reactions in pregnancy are the skin tests in cancer with tumor juices, on the basis that the proteins of new cancer cells may effect sensitization of the host.

Ransohoff⁹ has reported that guinea-pigs passively sensitized with 0.1 c.c. serum from individuals with advanced cancer can be thrown into an anaphylactic reaction about ten days later by the intraperitoneal injection of 3 to 5 c.c. of blood-serum from individuals with cancer. Presumably under these conditions serum in cancer carries both antibody and antigen. The author concluded that the test may prove of diagnostic value, inasmuch as it occurred regularly in cancer and had a margin of error of not more than 8 per cent. Pfeiffer¹⁰ and Ranzi¹¹ had previously reported positive reactions employing guinea-pigs actively sensitized by injections with tumor juices.

Further reports on work of this kind do not appear to have been made, and the methods of passive and active anaphylactic tests for cancer have not been definitely established.

Skin tests of a different character have been employed by Elsberg, Neuhof, and Geist¹² in the diagnosis of cancer, consisting of the subcutaneous or intracutaneous injection of a few drops of a 20 per cent. suspension of the washed corpuscles of a normal healthy person. This test is not allergic, but simply a test *in vivo* for the isohemolysins sometimes developing in

¹ Münch. med. Wchn., 1911, lviii, 1705.

² Jour. Med. Research, 1914, 24, 425.

³ Archiv. f. Gyn., 1911, 92.

⁴ Jour. Obstet. and Gyn. of Brit. Empire, 1911, 19, 253.

⁵ Münch. med. Wchn., 1914, lxi, 1115.

⁶ Münch. med. Wchn., 1914, lxi, 1115.

⁷ Amer. Jour. Obstet., 1915, 71, No. 6.

⁸ Amer. Jour. Obstet., 1914, 70, 884.

⁹ Jour. Amer. Med. Assoc., 1911, 57, 103; 1913, 61, 8.

¹⁰ Ztschr. f. Immunitätsf., 1909-10, 4, 458.

¹¹ Ztschr. f. Immunitätsf., 1909, 2, 12.

¹² Amer. Jour. Med. Sci., 1910, 139, 264.

cancer. The authors reported 89.9 per cent. positive reactions among persons with cancer and about 6 per cent. reactions among controls.

Warfield,¹ however, observed only 32.4 per cent. positive reactions and concluded that the reaction was without value in the early diagnosis of cancer; similar results were observed by Risley.² Lisser and Bloomfield³ observed positive reactions in 62 cases of a series of 158 and negative reactions in about 90 per cent. of controls.

¹ *Archiv. Int. Med.*, 1911, 7.

² *Boston Med. and Surg. Jour.*, July, 27, 1911.

³ *Bull. Johns Hops. Hosp. Bull.*, 1913, 23, 356.

CHAPTER XXXII

TREATMENT OF HUMAN ALLERGIES

Principles.—The *prophylactic treatment of allergy* embraces methods for the prevention of allergic reactions. This may be accomplished by (a) removing the intake of the allergen (exciting substance); (b) by removal of the allergic antibodies from the sensitized cells; (c) by the administration of drugs tending to depress allergic cells or the effects of allergic shock or by a combination of these.

After the allergic reaction has occurred as in “serum sickness,” asthma, etc., remedial measures are directed to the *treatment of allergic shock*; this treatment is usually symptomatic, as the administration of adrenalin chlorid for the relief of urticaria. It may be, however, a form of specific treatment, as the administration of pollen extracts for the relief of hay-fever during an attack by the process of desensitization.

The prevention of allergy and the specific treatment of allergic reactions depends upon the process of *desensitization*; this refers to the exhaustion of allergic antibody from sensitized cells by *gradually* and slowly bringing the allergen or exciting substance into contact with these cells. Probably a series of allergic shocks occur, but ill effects are avoided by rendering these of a mild character. Exhaustion of antibody results in anallergy or desensitization of the cells; the process generally requires considerable time and the administration of a large number of doses of allergen and particularly in cases of natural allergy, as hay-fever, the dust asthmas, and food allergies.

This process of desensitization is generally a specific process, that is, the antibody is exhausted by the administration of its specific allergen. But partial desensitization can sometimes be brought about by the administration of a non-specific substance. For example, in allergy to horse-serum the injection of beef-serum may protect against a subsequent injection of horse-serum; in the opinion of some observers the injection of a pollen extract to which allergy does not exist may partially desensitize against the pollen to which sensitization does exist.

Desensitization is not anti-anaphylaxis. The latter term as originally defined by Besredka and Steinhart meant desensitization, but, as discussed in Chapter XXVIII, we have adopted Weil’s definition to cover that state of anallergy in which the sensitized cells are protected against the exciting agent by the presence of antibody in the blood. These free antibodies are supposed to neutralize the allergen before it reaches the cells. According to this definition anti-anaphylaxis is really a state of extreme sensitization with an excess of antibody and probably is of exceptional occurrence in human beings, inasmuch as the demonstration of the presence of these free or excess antibodies by injecting human blood into a lower animal (passive sensitization) is only occasionally successful in serum allergy.

The theoretic considerations of specific and non-specific desensitization and anti-anaphylaxis are given in Chapter XXVIII. In this chapter the practical aspects and methods of prophylaxis and treatment of human allergies will be considered.

PROPHYLACTIC TREATMENT OF SERUM ALLERGY

This refers almost entirely to the treatment of allergy to horse-serum since immune horse-sera are widely employed for the prophylaxis and treatment of some of the infectious diseases, and notably diphtheria, tetanus, and meningococcus meningitis.

Prevention of Sensitization.—No efficient method of preventing sensitization of human beings by horse-serum has been discovered, although this is a very important problem worthy of investigation.

Besredka¹ states that heating therapeutic sera to 56° C. for an hour on four days in succession renders the serum less sensitizing. This method is practised at the Pasteur Institute, and Besredka is of the opinion that it "explains why in France serum accidents have always been relatively rare, and why in the small number of cases (13 per cent.) in which they occur they are not of such a serious nature as they are in countries where sera are not heated."

Cases Requiring Desensitization.—Not all persons who have received injections of normal or immune horse-serum become sensitized or require desensitization before a subsequent injection may be given.

In the majority of instances serum is given subcutaneously or intramuscularly for the prophylaxis and treatment of diphtheria or the prophylaxis of tetanus, and while serum sickness may follow, desensitization is not absolutely required. *When serum is given intravenously and especially to individuals who have received serum on a previous occasion or when serum is to be given by any route and even by simple subcutaneous injection to asthmatics or individuals known or suspected to be hypersensitive, desensitization is advisable and necessary.*

The writer has formulated the following rules which have proved quite satisfactory in practice for guidance in this perplexing problem:

1. Horse-serum should never be given to "horse asthmatics" by any route of parenteral injection—subcutaneous, intramuscular, intrathecal, or intravenous—without previous desensitization and very special precautions.

Horse asthma is usually a natural allergy, that is, acquired by unknown means. There are some cases on record, however, where this extreme sensitization has apparently resulted from a previous injection of horse-serum.

Some cases of horse asthma yield skin reactions to the proteins of horse hair and dandruff and not to serum, but the majority react to serum as well, and all are extremely bad risks. Desensitization of these persons to the point where an adequate dose of serum may be safely given is a tedious process and frequently impossible by our present methods. Horse-serum should be avoided and it is very much desired that our manufacturing laboratories render available diphtheria and tetanus antitoxins and anti-meningococcus serum by the immunization of cattle for use in these individuals.

If horse-serum is given at all, even after attempts to secure desensitization, the injections should be subcutaneous and the preliminary injection of 1/50 grain of atropin is advisable. After the injection the patient should be carefully watched and treated as described on p. 662 if a reaction occurs.

All cases of asthma due to other proteins are risks and especially if serum is given intravenously and intrathecally (intraspinal injection); likewise cases of thymolymphaticus. At least skin tests should be done and desensitization

¹ Anaphylaxis and Antianaphylaxis. Translated by Gloyne, C. V. Mosby Co., St. Louis, 1919.

practised if positive reactions are observed. Subcutaneous injections are the least dangerous if serum must be administered.

2. A skin test should be made before serum is given intravenously even though it is the first injection of serum the patient has ever received. Even if the reaction is negative it is good practice to give 1 c.c. of the serum subcutaneously one hour before the intravenous injection. If the reaction is positive several preliminary subcutaneous injections should be given.

Serum given intravenously is brought into sudden contact with the cells and conditions are perfect for eliciting shock if the cells are sensitized. It is to be remembered, however, that ill effects may be due to non-specific protein shock, and as these may not be allergic, desensitization may not prevent such symptoms as fever and chills.

The same applies to the administration of serum by intraspinal injection, but the danger is less.

3. When an individual has been injected with horse-serum some weeks, months, or years previously and serum is to be administered again by intravenous or intraspinal injection, preliminary desensitization is always advisable regardless whether or not skin tests are made. Skin tests, however, are advisable because if positive reactions are observed desensitization should be more thorough than if they are negative.

4. When serum is to be given by subcutaneous or intramuscular injection, skin tests and desensitization are not necessary if the patient is not asthmatic and if it is the first injection. "Serum disease" may follow five to twelve days after the injection, but is not dangerous.

Occasionally severe allergic reactions of collapse, dyspnea, tachycardia, and fall in blood-pressure follow the subcutaneous injection of serum into individuals who have received an injection one to six or eight weeks previously; for this reason a desensitizing injection of 1 c.c. of serum one hour previously is advisable before the administration of the balance of the serum by subcutaneous or intramuscular injection.

The following table summarizes this subject in a brief manner:

DESENSITIZATION

Not required	1. When patient is not asthmatic, has never before received serum, and when the injection is to be subcutaneous, intramuscular, or subthecal.
	2. When patient is not asthmatic, but has received serum before, and the injection is to be subcutaneous or intramuscular.
Advisable....	1. When serum is to be given for the first time intravenously to an individual who has not received an injection of serum on a previous occasion.
	2. When serum is to be given intravenously or intrathecally to an individual who has received serum on a previous occasion.
	3. When serum is to be given subcutaneously, intrathecally, or intravenously to an individual who is an asthmatic and regardless of whether or not serum has been previously administered.

Methods for Specific Desensitization.—Unfortunately, methods proving satisfactory for the desensitization of guinea-pigs and other of the lower animals do not desensitize human beings equally well. Ordinarily four methods may be employed according to circumstances, as follows:

(a) *Precautionary Desensitization; Individual Not Asthmatic and Skin Test Negative.*—Sterile horse-serum (either normal serum or the immune serum to be employed) should be injected subcutaneously in dose of 1 c.c. at least one hour and preferably two hours, before the balance of serum is administered. If the serum is to be given intravenously the first few cubic centimeters should be injected very slowly. Desensitization persists for from six to eight hours to several days.

The following methods are modified after those of Besredka when serum is to be injected subthecally: *Method A*, Give 2 c.c. by intraspinal injection and balance (20 to 30 c.c.) one or two hours later. *Method B*, Dilute 2 c.c. serum with 18 c.c. of sterile saline solution in a 20 c.c. syringe and give 1 c.c. (=0.1 c.c. undiluted serum) intravenously; while the needle is still in the vein wait three minutes, and if there are no symptoms inject 5 c.c. Wait two minutes, and if there are no symptoms inject the balance of 14 c.c. Serum may now be given subthecally at any stage of the illness.

(b) *Required Desensitization; Individual Not Asthmatic, but Skin Test is Positive and Serum is to Be Given Subcutaneously or Intramuscularly.*—Usually the above subcutaneous method is sufficient or the following: subcutaneous injection of 0.5 c.c. serum followed in one hour by 1 c.c. Two hours later the balance of serum by subcutaneous or intramuscular injection.

(c) *Required Desensitization when the Individual is Not Asthmatic, but the Skin Test is Positive and Serum is to Be Given Intravenously or Intrathecally.*—In these cases desensitization is tedious and time consuming inasmuch as it should be more thorough; the following method is employed in the Rockefeller hospital.¹ Subcutaneous injection of 0.025 c.c. serum followed at one-half-hour intervals by 0.05, 0.1, 0.2, 0.4, and 1 c.c. If a mild reaction follows the last injection, it should be repeated once or twice; if no reaction follows the injections may be continued by intravenous injection at one-half-hour intervals beginning with 0.1 c.c. and doubling the dose at each injection. If a general reaction occurs, as cyanosis, dyspnea, or increased rapidity of the heart rate supervenes, the injections should be suspended for two to four hours, depending upon the severity of the reaction, and then be resumed, starting with the same dose as that producing the reaction. After 25 c.c. of serum have been given in these small doses, after a lapse of four hours, 50 c.c. may be given, followed by the regular dose six to eight hours later. Fortunately, one rarely is called upon to employ this measure; at the Rockefeller Hospital it has been found necessary to desensitize in this way only 2 or 3 patients in a series of over 150 cases.

In addition to these attempts at specific desensitization medicinal means may be employed for the prevention of shock as described below.

(d) *Required Desensitization when the Individual is a Horse Asthmatic.*—The administration of serum by any route of injection is dangerous, and, if possible, should be avoided. This is particularly true if skin tests with serum are positive; if skin tests with serum are negative the danger is less. Fortunately, these cases are uncommon. If, however, an individual requests desensitization for the relief of allergic horse asthma, bronchitis, and rhinitis or in preparation for the time when serum injections may be required, the method described on p. 734 may be employed. But if serum is subsequently administered for prophylactic or therapeutic purposes at a later date, the injections should always be subcutaneous and started with 0.1 c.c. followed at one-half-hour intervals by gradually increasing amounts. Atropin sulphate and adrenalin may be administered in addition as precautionary measures, the method being described in the following section.

Medicinal Prophylaxis of Anaphylactic Serum Shock.—Two drugs have been found useful in the prevention of anaphylactic serum shock of guinea-pigs, namely, *atropin sulphate* and *adrenalin chlorid*.

As first shown by Auer² and confirmed by Anderson and Schultz,³ Karsner and Nutt,⁴ atropin prevents or relieves the active bronchoconstriction

¹ Monograph No. 7 on Acute Lobar Pneumonia, 1917.

² Amer. Jour. Physiol., 1910, 26, 439.

³ Proc. Soc. Exper. Biol. and Med., 1910, 7, 32.

⁴ Jour. Amer. Med. Assoc., 1911, lviii, 1023.

induced by serum anaphylaxis; similar effects were observed by Bredl and Kraus,¹ with peptone, and by Baehr and Pick,² with both peptone and histamin. Friedberger and Mita³ were unable to demonstrate any appreciable effects of atropin upon serum anaphylaxis. However, Hanzlik and Karsner⁴ have again confirmed earlier work and found that atropin in guinea-pigs in the dosage of 0.01 mgm. per gram of body weight can completely prevent the toxic effects produced by the intravenous injection of beef-serum and peptone.

The effects of adrenalin are more complicated and not so certain, depending in part on the functional state of the bronchiolar musculature and in part on the time of administration, since the action is of short duration. Hanzlik and Karsner found that adrenalin in dosage of 0.0005 c.c. of 1 : 10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in guinea-pigs. Pelz and Jackson⁵ also found adrenalin to relax the constricted bronchi in anaphylactic shock if injected early.

In the prophylaxis of serum anaphylactic shock of man atropin sulphate in dose of 1/50 grain may be given an adult by subcutaneous injection about one-half hour before the serum is administered. Adrenalin chlorid in dose of 1 c.c. of a : 1000 dilution should be in readiness for injection if necessary.

Sodium chlorid has been found by Langer,⁶ Brodin, Richet, and Girons⁷ as a prophylactic for anaphylaxis in the lower animals, the latter administering 0.8 gm. per kilogram of body weight to dogs. More recently Sicard and Parof⁸ have advised the intravenous injection of human beings with 1 gm. of sodium carbonate in 30 to 40 c.c. of water fifteen minutes before the injection of serum, as a means for preventing precipitation *in vivo*, which they maintain produces anaphylactic shock.

According to Besredka ether narcosis reduces the tendency to anaphylactic shock as discussed in a previous chapter. Under very exceptional circumstances it may be advisable to reduce the chances of anaphylactic shock in human beings by light etherization.

The **treatment of serum anaphylactic shock** has been previously described on p. 662.

TREATMENT OF POLLEN ALLERGY (HAY-FEVER)

Soon after the allergic nature of hay-fever was established on a scientific basis Noon⁹ in 1911 reported the results of his investigations in the laboratory of Sir Almroth Wright on specific treatment with subcutaneous injections of pollen extracts in minute doses. He injected extracts of timothy pollen and controlled the doses by an ophthalmic reaction. At the same time and independently Koessler¹⁰ was working along the same lines. Curtis,¹¹ Dunbar,¹² and others had previously used plant and pollen extracts, but none had achieved results sufficiently promising or reliable to encourage a continuation of the work. Since then numerous reports have been made on the desensitization treatment of hay-fever by injection of pollen extracts by Freeman,¹³

¹ Zentralb. f. Physiol., 1910, 24, 258.

² Arch. f. exp. Path. Pharm., 1913, lxxiv, 65.

³ Ztschr. f. Immunitätsf., 1911, 11, 501.

⁴ Jour. Pharm. Exper. Therap., 1920, 14, 425.

⁵ Jour. Pharm. Exper. Therap., 1918, 11, 173.

⁶ Münch. med. Wchn., 1912, 59, 2545.

⁷ Rev. d. méd., Paris, 1920, 37, 7.

⁸ Bull. d. l. Soc. med. d. Hôp., 1921, 45, 60.

⁹ Lancet, 1911, 1, 1572.

¹⁰ Forchheimer's Therapeutics of Internal Diseases, 1914, 5, 671.

¹¹ New York Med. News, 1900, 37, 16.

¹² Deut. med. Wchn., 1911, 37, 578.

¹³ Lancet, 1911, 2, 814; *ibid.*, 1914, 1, 1178.

Clowes,¹ Lovell,² Lowdermilk,³ Ulrich,⁴ Manning,⁵ Cooke,⁶ Goodale,⁷ Hitchens and Brown,⁸ Scheppegrell,⁹ Walker,¹⁰ and others.

The Etiology of Hay-fever.—Hay-fever or "rose cold," as ordinarily understood, is caused by the pollens of different grasses and plants. The effects are due primarily to three causes: (a) Sensitization to the proteins of these pollens; (b) direct irritation of the conjunctiva and the mucosa of the respiratory tract by the pollens, and especially those of the ragweed class, which are furnished with spicules, and (c) bacterial infection and especially in ragweed pollinosis, owing to direct injury of the involved mucous membranes and subsequent infection. In addition to these some pollens apparently contain toxic substances, the hay-fever "toxin" of Dunbar. Too much work has been done with these substances to deny that they may be present and even contribute to the lesions and symptoms of hay-fever, but the essential nature of hay-fever appears to be an allergic sensitization to the proteins of various pollens. It is well known that solutions of pollen proteins become toxic upon hydrolysis, and it is possible that a similar change may take place on the mucous membranes during the hay-fever season with the production of toxic substances.

Scheppegrell¹¹ has divided these pollens into two classes: (a) The spiculated, low in protein and causing direct hay-fever, the severity of the attack and its duration depending mainly on the number of pollen grains in the atmosphere and the length of the spicules. The ragweeds form the type and the principal cause of this form of hay-fever. (b) The unspiculated, high in protein and causing indirect hay-fever, the severity of the attack and its duration depending on the amount of protein and number of pollens in the atmosphere. The grass pollens are a type.

A very large number of pollens have been identified as causing hay-fever and this greatly complicates diagnosis by skin tests and treatment by specific desensitization. Scheppegrell states that pollens without spicules and with an inappreciable amount of protein are innocuous, but there remains a discouragingly large number which may cause hay-fever. Walker states that these vary in different parts of the country and that it is advisable to study with particular care the hay-fever producing vegetation in different localities in order to secure pollens for diagnosis and treatment. In New England he found that the chief causes were the pollens of ragweed, timothy, and June grass; occasionally rose, red top grass, and various trees.

In addition to the pollens of grasses and plants as causes of hay-fever, Walker has observed 12 patients who were sensitive to the pollens of trees: oak, maple, willow, pine, poplar, ash, and to apple blossoms. These pollinate from April to early June. In addition to these Walker believes that substances from the leaves of trees, like the fine hairy growths on the under surfaces of the willow and plantain leaves, may cause hay-fever.

Selfridge¹² has made a study of the hay-fever pollens in California, and Watson and Kibler¹³ for Arizona and the southwest. The hay-fever seasons

¹ Proc. Soc. Exper. Biol. and Med., 1912-13, 10, 69.

² Lancet, 1912, 2, 1716.

³ Jour. Amer. Med. Assoc., 1914, 63, 141.

⁴ Jour. Amer. Med. Assoc., 1914, 62, 1220.

⁵ Jour. Amer. Med. Assoc., 1915, 64, 655.

⁶ Laryngoscope, 1915, 25, 108.

⁷ Boston Med. and Surg. Jour., 1915, clxxiii, 42.

⁸ Jour. Lab. and Clin. Med., 1916, 1, 457.

⁹ New York Med. Jour., 1918, 1016; *ibid.*, 1919, cix, 793; Medical Record, 1918, 141.

¹⁰ Arch. Int. Med., 1921, 28, 71.

¹¹ Jour. Amer. Med. Assoc., 1916, 67, 861.

¹² Californ. State Jour. Med., 1918, 16, 164. ¹³ Jour. Amer. Med. Assoc., 1922, 98, 719.

vary in different sections of the country and different plants are responsible. These may be summarized as follows:

- | | | |
|---|---|--|
| (a) Spring hay-fever
(April, May, June, and July) | } | In the East: Principally the pollens of grasses, as timothy, June, and red-top.
In the Middle West: June grass (blue grass) and sweet vernal grass.
In all sections: The pollens of early flowering trees (poplar, birch, etc.). |
| (b) Summer hay-fever
(July, August, and September) | } | Goose foots, amaranths, and docks. |
| (c) Fall or autumnal hay-fever | } | In the East: Principally ragweed.
West of Rocky Mountains: Principally the artemisias.
Southwest: Amaranths. |

East of the Mississippi river timothy pollen is the usual cause of spring fever and ragweed pollen the usual cause of fall or autumnal fever.

Why the majority of human beings and apparently the lower animals escape hay-fever is unknown. The unfortunate sufferers do not usually present anatomic defects or predisposing pathologic changes to account for their affliction. Evidently a disposition to acquire the disease is inherited, as claimed by Vander Veer and Cooke; certainly the majority of patients exhibit a peculiar tendency to vasomotor disturbances of the skin and mucous membranes to different irritants ordinarily harmless for the majority, and in many instances other members of the family are similarly afflicted.

The Prevention of Hay-fever.—As Scheppegegrell¹ has stated for the American Hay-fever Prevention Association, it is important to educate the public on the relation of weeds and plants to hay-fever for the purpose of securing co-operation for their removal; at present neglect and careless cultivation are a source of disease and discomfort to a large class of sufferers. Undoubtedly a campaign of this kind would produce immediate results, even if it did nothing more than to teach recognition of the ragweed followed up by the removal of these as far as possible from vacant lots and gardens in different communities.

Patients may help themselves by avoiding proximity to the vegetation to which they are sensitized, and by means of skin tests the physician can render great aid in determining the pollens to which sensitization exists.

Patients who are sensitive to ragweed should be warned not to smell of goldenrod, golden glow, sunflower, poppy, aster, chrysanthemum, and the like that pollinate during the ragweed season. Those sensitive to grasses should avoid contact with clover, lilies, daisy, dandelion, rose, lawn grass, orchard grass, corn, and the like that pollinate during the grass season (Walker).

Removal to a locality where the pollens do not occur, as the Adirondacks and White Mountains for the eastern states, is usually followed by immediate relief. A sea voyage or residence in a high locality with dry mountain air during the hay-fever season is particularly desirable, but only a small percentage of sufferers can avail themselves of these means of escape. Sometimes a sojourn at the seaside brings relief and particularly if the neighboring country is free of ragweed.

Value of Preseasonal Desensitization.—Desensitization by means of a series of subcutaneous injections of an extract of the pollen or pollens to which sensitization exists is the only means offering hope for relief for the majority of hay-fever sufferers.

¹ Jour. Amer. Med. Assoc., 1916, 66, 707.

The results obtained by preseasonal desensitization have been fairly satisfactory. It would appear that many patients are helped in that the seasonal attack is less severe, others are not helped at all, and a few are apparently entirely relieved for the season, especially those who have had slight rather than severe attacks. The author believes that the principle of desensitization is correct and that as technic is improved upon the results will be more satisfactory.

Unfortunately, many difficulties are encountered. In the first place, desensitization of the acutely and severely sensitized individual cannot be accomplished by a few injections of the pollen allergen; an error in the past has been dependence upon six or less injections. I am convinced that a long series of injections are necessary and that for the prevention of autumnal fever ordinarily starting about August 15th, desensitization should begin in January. In severe cases and especially those complicated by asthma, desensitization should be continuous for a year or two to bring about gradual relief and possibly complete desensitization.

Second, it may not be possible to determine by skin tests the nature of the sensitization unless a large number of pollens are available; this is especially true of the early or spring types of hay-fever which may be caused by so many different pollens. An extensive series of pollens are required for skin tests and for the preparation of extracts for injection; for this reason both diagnosis and treatment can be undertaken in a proper manner by only a few physicians.

Naturally the question arises whether injections of a pollen extract to which a patient is not sensitive will induce allergic sensitization and thereby prove harmful. According to experiments on the lower animals this apparently does not occur, but inasmuch as the hay-fever sufferer inherits an increased tendency to acquire sensitization, I believe that great care should be exercised and that the only pollen or pollens to be injected are those which produce positive skin reactions.

Some writers have claimed that preseasonal desensitization may intensify the symptoms of hay-fever during the seasonal attack. Doubtless this may occur if too large doses are given just prior to the attack, but I have never seen ill effects attributable to preseasonal desensitization. Of course it is well known that, owing to climatic and other conditions, the symptoms vary in severity in different years, and this is to be borne in mind in this connection.

A question of considerable practical importance concerns the possibility of non-specific desensitization to several different pollens by injecting an extract of one pollen. A few authors claim that this may occur, and that if a patient is sensitive to several pollens, that desensitization to all may be effected in part, at least, by a series of injections of an extract of a single pollen. I have no accurate data to contribute on this subject, but probably this non-specific desensitization is possible to a limited degree with the pollens of grasses very closely related botanically. In serum allergy a certain amount of non-specific desensitization of animals has been observed by injections of a heterologous serum, but the degree of desensitization is slight and of short duration. Possibly a similar action may follow with pollen allergens, but very probably the degree of desensitization by a heterologous pollen is likewise slight and of short duration.

Success in the specific prevention and treatment of hay-fever by desensitization appears to depend mainly upon the following factors:

1. Accurate diagnosis of the sensitization. This refers to a complete diagnosis, inasmuch as some patients are allergic to more than one kind of

pollen. Skin tests are undoubtedly valuable in this connection, as so many patients do not know exactly on the basis of experience to which pollen or pollens they are hypersensitive.

2. Prolonged desensitization with *active* allergens. Deteriorated allergens are to be avoided. Doses must be adjusted to bring about desensitization without producing ill effects.

Inasmuch as the number of sufferers is large, means must be provided for affording relief by desensitization. For this purpose I believe that the manufacturing biologic laboratories should co-operate with the medical profession along these lines:

(a) Prepare an extensive number of pollens from vegetation in different parts of the country and render these available for skin tests.

(b) Be prepared to put up special antigens for desensitization for individual patients upon request by physicians.

In this manner the pollens may be preserved in powder form against deterioration and the antigen made up fresh from time to time and according to the needs of individual patients instead of trying to meet the situation by stock allergens which have not yielded heretofore the best possible results.

Preparation of Pollen Allergens.—Plants are collected during their season and as soon as some of the pollen pods begin to open. The pollen granules are collected and dried. In this condition pollens do not deteriorate; according to Goodale they have been kept twenty-five to thirty years without any loss in antigenic activity.

Wodehouse¹ has described the following method for collection: "The flower heads are stripped from young plants, just coming into bloom, and after being allowed to become almost dry, are crushed in a mortar with several volumes of carbontetrachlorid. After thoroughly macerating, the liquid is strained off through muslin. Most of the pollen, liberated by crushing from the anthers, passes with the CCl_4 through the muslin and can be collected on filter paper and be washed with fresh CCl_4 , or with the same, after filtering out the pollen, to remove what pollen remains after the first washing.

"The CCl_4 can be used several times, or until it becomes impregnated with oils, etc., when it should be replaced or distilled to remove the impurities.

"This method is very rapid and was found to yield many times more pollen than any other known to the writer. Experience has also shown that this treatment in no way interferes with the anaphylactic properties of extracts prepared from pollen procured in this way; but in order to get the best results only young plants, taken when the flowers are just opening, should be used, and these collected away from dusty roadsides."

After the pollens have been collected and dried different methods may be employed for the preparation of the material for skin tests and injection. Dunbar, Noon, and Freeman extracted the pollen with distilled water, aided by repeated freezing and thawing; Clowes precipitated the pollen with acetone and extracted with water. Koessler employed 8.5 per cent. salt solution followed by precipitation with 10 volumes of alcohol. Goodale soaked the pollens in 15 per cent. alcohol.

Koessler has emphasized that water extracts were unstable and likely to deteriorate in a few weeks. Clock² has advocated extraction with a solvent composed of $66\frac{2}{3}$ per cent. glycerol and $33\frac{1}{3}$ per cent. saturated sodium chlorid solution.

¹ Boston Med. and Surg. Jour., 1916, clxxiv, 430.

² Jour. Infect. Dis., 1917, 21, 523.

An excellent method is to collect the granules either mechanically or by the method of Wodehouse and macerate them in 10 per cent. sodium chlorid solution, which has been rendered faintly alkaline with sodium carbonate. The extract is now filtered, the filtrate dialyzed free of salts, precipitated with acetone, and the precipitate dried with anhydrous acetone. The powder so obtained keeps well and may be employed for skin tests and the preparation of allergen for subcutaneous injection as follows:

For these purposes the method of Walker is satisfactory: To 0.5 gm. the dry pollen is added 44 c.c. of sterile physiologic saline solution and the mixture is shaken thoroughly at frequent intervals for twenty-four hours, after which 6 c.c. of absolute ethyl alcohol are added. The mixture is again thoroughly shaken for twenty-four hours, centrifugalized at high speed, and the supernatant fluid removed and saved. This 1 : 100 solution of pollen extract is used as stock, and higher dilutions, as 1 : 500, 1 : 1000, 1 : 5000, and 1 : 10,000, prepared as required with a diluent of 12 per cent. alcohol in saline solution. These solutions are used for skin tests and treatment, and with the addition of a small crystal of thymol are said to keep for many months in a cool place.

A second method is to grind 1 gram of the dried pollen with powdered glass until few or no intact granules remain. This mass is then extracted with 100 c.c. of N/100 sodium hydroxid in saline solution containing 0.5 per cent. phenol by shaking mechanically for two hours followed by three days in a refrigerator with occasional shakings, and finally by several hours in a shaking machine. The material is then filtered through paper and finally a Berkefeld N or Mandler filter. Coca¹ employs an alkaline saline solution containing 0.4 per cent. phenol described on p. 681 for preparing extracts for skin tests and treatment and has described methods for the collection of pollens.

These extracts may be standardized according to the content of Kjeldahl nitrogen as suggested by Cooke and Vander Veer. For routine use five strengths have been employed containing, respectively, 0.01, 0.1, 0.5, 1, and 10 mg. of nitrogen per 100 cubic centimeters.

Standardization may be effected by the complement-fixation test employing the immune sera of rabbits, which have been injected with a gradually increasing amount of pollen extract. A unit of antigen is the smallest amount giving complete fixation with a given amount of serum. A *pollen unit* is equivalent approximately to 1/20 of a unit of antigen.

For skin tests about 60 pollen units may be employed (0.01 c.c. of a solution of proper strength). For desensitization treatment begins with the injection of about 2 units and ends with 600-1000 units.

Walker's Method of Preseasonal Desensitization.—Walker advises skin tests to determine the *degree* of sensitization by testing with the 1 : 100, 1 : 500, 1 : 1000, 1 : 5000, and 1 : 10,000 dilutions of pollen described above; treatment is begun with the strongest dilution which fails to give a skin reaction. With the majority of patients he has found a more or less positive reaction with the 1 : 5000 dilution and begins treatment with 0.1 c.c. or 0.2 c.c. of the 1 : 10,000 dilution. Treatments are given subcutaneously once a week and each week the amount of the extract is gradually increased, so that as the treatment progresses, stronger and stronger dilutions are used, until with fourteen injections a dose of 0.25 c.c. of the 1 : 100 dilution is reached.

Author's Method of Preseasonal Desensitization.—As recently emphasized by Vander Veer² pollen extracts employed for desensitization and

¹ Jour. Immunology, 1922, 7, 163.

² Jour. Immunology, 1922, 7, 113.

treatment of hay-fever are frequently too weak for the majority of patients and that larger therapeutic doses and more active extracts should be employed; the writer is in thorough accord with these opinions and employs the following plan of treatment:

1. Skin tests are first conducted with powders of various purified pollens prepared as described above for the purpose of determining the pollen or pollens to which sensitization exists. When this has been used tests are repeated with 1 : 100, 1 : 500, 1 : 1000, 1 : 5000, and 1 : 10,000 dilutions of the pollen to determine the size of the first dose, which is 0.1 or 0.2 c.c. of the highest dilution just failing to produce a reaction. In cases of autumnal fever which are so frequently due to ragweed or when the history and previous tests indicates this, the skin tests are done at once with the dilutions.

2. The injections for desensitization are given subcutaneously once a week. When one pollen is employed the series is of 24 injections and is started about February 1st in order that all may be given by August 1st in the case of autumnal fever. If two or more pollens are employed, a series of 28 injections are given beginning in the early part of January.

In spring fever the injections are started in October or November in order that the series may be completed by the early part of May.

3. The proper protein or allergen is purchased or prepared for the patient by adding 0.1 gm. of the pollen yielding a positive skin reaction, to 8.5 c.c. sterile salt solution containing 0.25 per cent. tricresol. This mixture is extracted in an incubator for two days with frequent shakings; 1.5 c.c. absolute alcohol are now added and the extraction continued at room temperature for two days followed by mechanical shaking for at least two hours. This material is now thoroughly centrifugalized, the supernatant fluid removed, cultured for sterility, and stored in a refrigerator as the stock 1 : 100 dilution.

If skin tests are positive to two or more pollens, a mixture is made of these, the total amount of powder extracted being 0.2 gm.

4. Dilutions 1 : 10,000, 1 : 5000, 1 : 1000, and 1 : 500 are prepared as required in rubber-capped vials from the stock 1 : 100 solution, using tricresolized saline solution as the diluent, as follows:

1 : 10,000 dilution: 0.1 c.c. of 1 : 100+9.9 c.c. diluent.

1 : 5000 dilution: 0.1 c.c. of 1 : 100+4.9 c.c. diluent.

1 : 1000 dilution: 0.5 c.c. of 1 : 100+4.5 c.c. diluent.

1 : 500 dilution: 1 c.c. of 1 : 100+4 c.c. diluent.

5. Four injections of the 1 : 10,000 dilution are given: 0.1, 0.2, 0.4, and 0.8 c.c., followed by 4 doses of each of the 1 : 5000, 1 : 1000, and 1 : 500 dilutions in the same amounts. These 16 injections are followed by 8 more of the 1 : 100 dilution: 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.5, and 2 c.c. In case the solution is prepared by two or more pollens, at least two more injections of the 1 : 100 dilution are given, these being in doses of 1 c.c., or 1.5 and 2 c.c. if well borne. The last dose should be given at least two weeks before the expected attack.

If the treatment begins too late to give the entire series of injections before the expected attack, as many as possible should be given, and frequently the doses may be increased more rapidly, this depending upon whether or not reactions are produced. This may be accomplished by giving increasing amounts of 1 : 10,000 dilution every day, 1 : 5000 every other day, and 1 : 500 and 1 : 100 dilutions every three days.

Skin tests may be repeated after the last injection; if negative with the 1 : 100 and 1 : 500 dilutions it is likely that no symptoms will develop during the season.

The series should be repeated the following one or two years and especially in the asthmatic types. The duration of desensitization varies with different persons. Whenever the skin tests are positive to a 1 : 100 dilution of pollen, a series of desensitizing injections should be given.

Seasonal Treatment.—Some patients are apparently relieved during the attack by subcutaneous injections of the pollen extract, but great care must be exercised against injecting too large doses and especially the first dose. Skin tests should be conducted with 1 : 1000, 1 : 5000, 1 : 10,000, and 1 : 20,000, dilutions and treatment begins with the subcutaneous injection of 0.1 c.c. of the highest dilution failing to produce a skin reaction (usually 1 : 20,000). Subsequent injections should be increased, but the amounts vary with individual patients; the desired effect is relief from the symptoms. As a general rule the relief is of short duration and the injections may be cautiously given every three to five days.

A host of drugs have been advised. Adrenalin chlorid is probably best for affording relief from the nasal congestion. Two drops of the following mixture may be instilled into each nostril two or three times daily with a medicine-dropper or applied with an atomizer:

Adrenalin chlorid (1 : 1000).....	6 c.c.
Rose-water.....	24 c.c.
Antipyrin.....	1 gm.

Adrenalin chlorid (1 : 1000) may be injected intramuscularly in dose of 0.5 c.c. for the relief of asthma, but the effects are of short duration. The subcutaneous injection of atropin sulphate may also prove helpful.

Treatment with Bacterial Vaccines.—During the hay-fever attack treatment with subcutaneous injections of autogenous bacterial vaccines are sometimes beneficial, and especially in ragweed hay-fever. Lowdermilk,¹ Strouse and Frank,² and Medalia³ have noted beneficial effects and Walker states that autogenous nasal vaccine or a mixed streptococcus stock vaccine given during the hay-fever attack benefits somewhat an occasional patient.

Autogenous vaccines are preferred; cultures should be made from both sides of the nose and preferably on blood-agar in order to facilitate the growth of pneumococci and streptococci if present, as well as the staphylococci, which usually are present. The author prepares a mixed vaccine containing approximately 2,000,000,000 per cubic centimeter; the first dose is 0.1 c.c. by subcutaneous injection. Subsequent injections are gradually increased and given at intervals of five to seven days. The injections may be given together with pollen extract or at alternate times. A further description of vaccine therapy is given in Chapter XL.

The preseasonal use of bacterial vaccines has been used by Strouse and Frank with benefit in some cases.

Treatment with Sera.—Within recent years treatment with Dunbar's antiserum (pollantin) and Weichhardt's cattle serum (graminol) have fallen into disuse. Dunbar's serum is prepared by the immunization of animals with pollen extracts and is marketed in various ways, of which a dried serum diluted with lactose and a fluid serum, are mostly employed.

The serum is used locally and usually just before and during the attack for the relief of symptoms. A small amount of the powder is dropped into each eye and insufflated into both sides of the nose. Large amounts are irritating and to be avoided. Best results have been reported by a daily

¹ Jour. Amer. Med. Assoc., 1914, 63, 141.

² Jour. Amer. Med. Assoc., 1916, 66, 712.

³ Boston Med. and Surg. Jour., 1916, 175, 201.

dose of the powder into both sides of the nose several days before the expected attack.

The early reports on the use of this serum were very enthusiastic. The German Hay Fever Association reported a full success in about 25 to 35 per cent. of all cases, relief in 25 per cent., and no benefit or aggravation in 40 to 50 per cent.

As stated, "graminol" is nothing more than the serum of cattle collected during the flowering of grasses. Apparently it has yielded as good results as pollantin.

Both of these sera were proposed for the neutralization of a supposed pollen toxin. As previously stated, pollens apparently contain toxic substances for which normal or immune serum may be antagonistic, but the chief effects are probably due to allergic sensitization and sera apparently confer little or no protection to the sensitized cells.

TREATMENT OF ALLERGIC ASTHMA AND VASOMOTOR RHINITIS

As stated on p. 653 a large number of different protein substances may cause allergic asthma; of these, the proteins of horse, cat and dog hair and dandruff and goose feathers, pollens, bacteria, face and dental powders, and certain foods are most important.

Vasomotor rhinitis or hay-fever-like attacks may be caused by the same substances or, as shown by Goodale,¹ by flowers that have no pollens and to which the individual is not sensitive. Some cases of rhinitis with which asthma may be associated, are caused according to Walker, by such mechanical agents as room dust, hay dust, and talcum; by chemical agents as soap-powders, lye, and ammoniacal fumes. Also by perfumes and other odors, as well as by sudden changes of temperature and especially upon retiring and arising.

Skin Tests as Guide to Treatment by Desensitization.—The first essential to treatment is accurate diagnosis, and while this may be possible by the history alone, skin tests have proved of particular value in this connection.

When skin tests are employed as a guide to treatment by desensitization, it is essential to use dilutions for the purpose of determining the degree of sensitization. Diagnostic tests may be conducted with the commercially prepared allergens or with the alcoholic extracts of different proteins as described on p. 681; those producing positive reactions should then be retested, according to Walker, with dilutions of purified proteins as 1 : 100, 1 : 1000, 1 : 10,000, 1 : 100,000, and 1 : 1,000,000.

Not infrequently a patient will react to more than one protein, but Walker is of the opinion that only that protein need be used for desensitization which yields a positive reaction with a dilution of 1 : 1000 or higher.

Treatment.—This may be of two kinds: (1) The removal of the exciting cause if this is possible; for example, the disposal of the cat if it is the exciting agent, or of feather pillows, omission of the food allergen from the diet, etc. (2) Desensitization by weekly injections of an extract of the protein or proteins.

Exclusion of the exciting agent or agents is of the utmost importance and may be sufficient. When this cannot be accomplished or is incomplete, as, for example, in horse asthma when street dust may carry sufficient allergen, desensitization is indicated.

We are greatly indebted to Walker² for his brilliant investigations and

¹ Boston Med. and Surg. Jour., 1918, 179, 293.

² Jour. Med. Research, 1917, 36, 423. Archiv. Int. Med., 1918, 22, 466; *ibid.*, 1919, 23, 220. Amer. Jour. Med. Sci., 1919, 157, 409.

pioneer work in this field. His results have been very encouraging and most favorable in the treatment of all forms of allergic asthma with the possible exception of the food asthmas.

Pollen Asthma (Hay-fever).—The plan of specific treatment is that described on p. 730. It is advisable to determine by quantitative skin tests the degree of sensitization and commence treatment with 0.1 c.c. of the highest dilution failing to give a positive reaction. These patients are usually extremely sensitive and desensitization requires much patience on the part of both physician and sufferer. I endeavor to give a course of 28 to 30 injections as previously described, and always advise a repetition of the series each year for two or three years.

Horse, Cat, Dog, and Fowl Asthma.—Quantitative skin tests are conducted with the purified proteins of the substance or substances yielding positive reactions in the preliminary tests and against which desensitization is to be tried if contact with the effluvia of the animal cannot be eliminated.

Only such proteins are employed as yield positive reactions in dilutions of 1 : 1000 or higher unless there is only one protein to deal with, in which case it is used even if positive only in 1 : 100. As a general rule, however, sensitization to this degree is not accompanied by asthma or rhinitis.

A stock 1 : 100 dilution of the protein in sterile tricesolized saline solution is prepared from which higher dilutions are made as required. As a general rule treatment begins with a 1 : 100,000 dilution, the injections being given subcutaneously at intervals of one week in ascending doses as follows: 0.1, 0.2, 0.4, 0.6, and 0.8 c.c. Sometimes the same dose must be repeated once or twice if severe local or asthmatic reactions are produced.

These injections are followed by five more of a 1 : 10,000 dilution and these in turn by 1 : 5000, 1 : 1000, and finally 1 : 100 dilutions.

Skin tests should be conducted at intervals and the desensitizing injections continued until negative with 1 : 100 dilutions.

The duration of desensitization cannot be stated; Walker has reported cases free of symptoms for periods up to two years, but very probably the duration will be longer depending upon the thoroughness of desensitization.

Horse-serum Asthma.—Sometimes a horse asthmatic will yield positive skin reactions to horse-serum as well as to the proteins of the hair and dandruff. Desensitization may be advisable as part of the desensitization to the horse in general and in relation to serum therapy.

Skin tests should be conducted with dilutions of normal horse-serum as 1 : 100, 1 : 1000, 1 : 10,000, 1 : 100,000, and even 1 : 1,000,000. Treatment begins with 0.1 c.c. of the highest dilution failing to produce a skin reaction.

These are prepared of sterile normal horse-serum diluted with sterile physiologic saline solution containing 0.25 per cent. tricesol.

The doses are gradually increased and not infrequently it is necessary to repeat the same dose several times until it no longer produces an unusual local reaction or attack of asthma. All injections are given subcutaneously at intervals of one week. After a dose of 0.8 c.c. of a dilution is reached the injections are continued with the next lower dilution until the 1 : 100 dilution is reached. This requires prolonged treatment, and while relief from rhinitis and asthma are to be expected, I do not believe that desensitization can be so complete as to render the injection of horse-serum safe for prophylactic or therapeutic purposes unless the serum is given cautiously by subcutaneous injection and never intravenously.

Food Asthma.—These cases are usually best treated by omission of the exciting food from the diet if this is possible. As a general rule sensitization to more than one food usually exists, and with some, as wheat, eggs and milk,

which enter into so many different foods, as pastries, gravies, and the like, omission may be a difficult matter.

Desensitization may be essential with certain foods, as egg, because the sufferer cannot always escape eating this food and especially when away from home when it may be present in foods where its presence is not suspected.

The food proteins for skin tests and desensitizing injections should be prepared of cooked foods if the allergen is usually ingested cooked, or of raw food if the food is generally eaten raw. Wodehouse¹ has described a method for the preparation of these proteins.

If desensitizing injections are to be given, quantitative skin tests should be made. Only those proteins yielding positive reactions with 1 : 500 or higher dilutions need be used. Treatment begins with 0.1 c.c. of the highest dilution failing to give a positive reaction. Injections are given once a week and in gradually ascending doses and dilutions until a 1 : 100 dilution is borne in dose of 1 c.c.

Bacterial Asthmas.—On p. 684 I described a method for conducting the skin tests with the bacteria recovered from the individual patient. In my opinion these autogenous vaccines are superior to stock bacterial allergens both for skin tests and treatment.

Only those bacteria producing positive reactions are used for desensitization. A stock vaccine of equal parts of the different bacteria is prepared so that each cubic centimeter has a total of 2,000,000,000 bacteria.

Treatment begins with 0.1 c.c. of a suspension of 1,000,000 bacteria per cubic centimeter and the doses are gradually increased. The injections are given subcutaneously. If no local or general reaction occurs, the succeeding doses may be given at intervals of three days until a local reaction results, after which the succeeding doses are given at intervals of one week. When 0.8 c.c. of this suspension is well borne the series is continued with suspensions of 10,000,000, 100,000,000, and 1,000,000,000 per cubic centimeter.

Treatment should be continued until there is complete symptomatic relief. Sometimes it is advisable to reculture at intervals and prepare fresh vaccines instead of continuing indefinitely with one vaccine. The duration of desensitization is variable, depending to some extent upon the thoroughness of treatment, but under favorable circumstances is likely to be periods of years.

A special effort should be made to remove foci of infection in the accessory nasal sinuses, apices of the teeth, in the tonsils or elsewhere, if these exist and are suspected as responsible for the sensitization. I have known of cases relieved by the extraction of teeth and drainage of apical abscesses caused by streptococci. This subject is further discussed in Chapter XL.

TREATMENT OF ALLERGIC DERMATITIS

Desensitization in ivy poison has been reported by Schamberg² following the internal administration of the tincture of rhus toxicodendron. The following mixture was employed:

Tincture of rhus toxicodendron.....	1 c.c.
Rectified spirit.....	5 "
Syrup of orange, sufficient to make.....	100 "

This mixture is taken about one month before the ivy season, beginning with 2 drops in water three times a day, and increasing by 3 drops each day

¹ Boston Med. and Surg. Jour., 1917, clxxvi, 467.

² Jour. Amer. Med. Assoc., 1919, 73, 1213.

until a dose of 3 teaspoonsful per day is reached. After this 1 teaspoonful per day in water should be taken throughout the season.

Desensitization is probably not complete, but frequently sufficient to prevent ivy dermatitis if direct handling of the plant is avoided.

During the attack of dermatitis Schamberg administers the tincture as above.

Strickler has reported similar results following the intramuscular injection of extracts and Alderson and Pruett¹ have reported favorably upon the treatment of poison oak by injecting 1 c.c. of extract intramuscularly. Almost invariably this was followed by relief of the local symptoms. Hannah² has reported the successful treatment of ragweed dermatitis by subcutaneous injections of extracts of ragweed pollen.

It would appear, therefore, that desensitization may be effectual in the prophylaxis and treatment of different forms of dermatitis venenata (poison ivy, poison oak, and poison sumac). It is first necessary to apply skin tests for diagnosis unless the patient is sure of the plant to which hypersensitivity exists.

Extracts for skin tests and desensitization may be prepared by macerating the leaves and extracting with 15 per cent. alcohol in physiologic saline solution containing 0.25 per cent. tricresol for a period of several days in the proportion of 10 grams of pulp of fresh leaves per 100 c.c. of alcohol. This 1 : 10 extract is then filtered and is ready for use.

Skin tests should be conducted with 1 : 10, 1 : 100, and 1 : 1000 or higher dilutions and treatment begun by the subcutaneous injection of 0.1 c.c. of the highest dilution failing to give a skin reaction. The injections are given once per week in ascending doses, as 0.1, 0.2, 0.4, and 0.8 c.c. of 1 : 1000 followed by 0.1, 0.2, 0.4, and 0.8 c.c. of 1 : 500 and 0.1, 0.2, 0.4, and 0.8 c.c. of 1 : 100. If skin reactions are positive with the 1 : 1000 dilution, but negative with 1 : 10,000, treatment should begin with the 1 : 10,000 dilution.

The series should start sufficiently early to finish a course of at least 12 to 16 injections before the season opens. Treatment should be continued until there are no skin reactions to the 1 : 10 extract.

A similar series may be given during the season, but skin tests should be conducted in the same manner for the purpose of determining the initial dose.

TREATMENT OF FOOD ALLERGY

As previously stated on p. 721 treatment consists of: (a) Withdrawal of the food or foods from the diet if this is possible; (b) desensitization by the internal administration of the purified food protein or the food itself in small and ascending amounts or by subcutaneous injection of the purified protein when this is possible.

Treatment by Elimination of Foods.—In some instances the number of foods to which a patient is susceptible is so large that treatment by withdrawal of the foods from the diet is impossible. It is important with such foods as milk and egg to avoid various foods prepared with them, as pastries, puddings, gravies, etc.

According to Schloss³ many patients with food allergy recover spontaneously and particularly cases of the congenital type due usually to milk or egg. The ill effects become less and less and ultimately the patient is able to eat the food in question without discomfort, but this does not occur in all cases.

¹ Californ. State Jour. Med., 1921, 19, 188.

² Jour. Amer. Med. Assoc., 1919, 72, 853.

³ Amer. Jour. Dis. Child., 1920, 19, 433.

This method of treatment is sufficient with such allergies as those caused by strawberries, buckwheat, etc., which are easily avoidable.

In eczemas and other conditions due to food allergies, elimination should be practised as far as possible without producing ill effects caused by deficient diet and undernutrition.

Treatment by Desensitization.—Desensitization by subcutaneous injection is complicated by the practical difficulty of securing the food proteins in solutions that are sterile and non-irritating.

These may be prepared of some foods as described on p. 682. Solutions (1 : 100) in sterile physiologic saline solution containing 0.25 per cent. tricrosol may be prepared of some of them; a faint alkalinity to aid solution does no particular harm.

Skin tests should be conducted with 1 : 100, 1 : 1000, and 1 : 10,000 or higher dilutions and treatment begun with the subcutaneous injection of 0.1 c.c. of the dilution failing to give a positive reaction. The injections are given once per week in ascending doses, as 0.1, 0.2, 0.4, and 0.8 c.c. followed by 0.1, 0.2, 0.4, and 0.8 c.c. of the next lower dilution, and so on until the skin tests become negative to the 1 : 100 dilution and preferably to the undiluted powdered protein applied to an abrasion.

Sometimes symptoms are produced by an injection; in this case the following dose should be smaller and subsequent doses cautiously increased.

Desensitization by injection is particularly serviceable with such foods as milk and eggs as cannot be easily avoided. In asthma due to food allergy and other incapacitating lesions, as angioneurotic edema and persistent urticaria, treatment of this kind may be imperative.

Desensitization by Feeding.—This form of desensitization is usually possible, the dried food or its proteins being administered in capsules in small ascending doses. These dried foods should be prepared of cooked foods if the food is usually ingested cooked, or of raw foods, if usually eaten uncooked. The capsules should be prepared of uncooked milk and eggs and the same is true of other foods eaten cooked or uncooked; as a general rule uncooked foods will desensitize more efficiently.

The doses administered should be small enough not to cause ill effects. Usually 0.001 or 0.0005 gm. of dried food, as dried egg-white or milk, diluted with starch, three times a day in capsules, are safe amounts with which to commence treatment. Each day the dose may be increased by one capsule until on the seventh day three are taken three times a day. In case symptoms are produced the dose is not increased until desensitization has occurred, when the dose is again increased. At the end of each week a new set of capsules are taken, each containing double the amount of the week previous. For example, if the treatment began with capsules containing 0.001 gm. of food, the second set would contain 0.002 gm. per capsule, the third set, 0.004 gm., and the fourth set 0.010 gm. per capsule. According to Schloss the patient should ultimately be able to ingest 15 to 30 gm. of the dried proteins or food in twenty-four hours.

Children under three years of age are usually unable to swallow capsules, and for them a solution must be used, but owing to direct contact with the buccal and pharyngeal mucosa the initial dose must be very small. Schloss has recorded a case of marked swelling of the tongue and lips of an infant one year of age after taking 1 drop of milk in a teaspoonful of water. In this case the initial dose was 1/20 drop of milk, three times a day.

Skin tests should be conducted from time to time as an index of the progress of desensitization. Thorough desensitization requires three to six months. After desensitization is accomplished, it is necessary for the

patient to take regularly a moderate amount of the food in order to prevent a return to sensitization. Sometimes urticaria or swelling of the lips develop in a desensitized individual, but in such cases it is only necessary to give treatment for a few days with larger amounts of food.

Schloss has treated 24 cases of milk allergy in this manner, with satisfactory results; all were markedly sensitive, but at the conclusion of the treatment were able to ingest the food without discomfort.

The duration of desensitization is variable. Schloss has followed 12 patients who have been desensitized from three to seven years. All of these patients are now quite desensitized and can eat, without discomfort, the foods to which they were sensitive.

TREATMENT OF DRUG ALLERGY

As a general rule these cases require no further treatment than elimination. It is well to bear in mind that drugs more or less in daily use, as powdered orris root in dental powders or creams, may be the cause of allergy. Diagnosis is of primary importance and is greatly aided by skin tests. As a rule the medicinal substance is easily eliminated. Most difficulty is experienced when drug allergies occur among physicians, dentists, and pharmacists who may be required to handle the drug in the course of their occupation.

Desensitization may be effected by subcutaneous injection or internal administration and may be required as in the treatment of malaria with quinin when the patient is allergic to this drug, as was true of a case reported by Heran and Saint Girons.¹ In their case the internal administration of 0.25 gm. of quinin sulphate produced dyspnea, fever, and general urticaria. Treatment was given according to the following plan:

	Gram.
First day 9.45 A. M.....	0.005
11.15 A. M.....	0.1
Second day at first.....	0.005
later.....	0.2
Third day.....	0.4
Fifth day.....	0.8
Sixth day.....	0.8
Seventh day.....	1.0

No symptoms followed any of these administrations of the drug, and the patient was cured of malaria.

A similar plan may be followed for desensitizing against mercurials, antipyrin, aspirin, arsphenamin (dermatitis), and other drugs. As a general rule skin tests are elicited by 10 per cent. solutions but seldom, in dilutions higher than 1 : 1000. If the drug is not irritating, subcutaneous desensitization may begin with 0.1 c.c. of the highest dilution just failing to produce a skin reaction. Subsequent doses should be given at intervals of a week and in gradually increasing amounts.

As a general rule desensitization can be effected by internal administration beginning with 0.001 gm. in capsules and after the same plan described above for desensitization in food allergy.

¹ Montpelier Med., 1917, 39, 669.

CHAPTER XXXIII

THE SCHICK TEST FOR IMMUNITY TO DIPHTHERIA

Natural Antitoxin Immunity to Diphtheria.—It is generally well known that not all persons exposed to diphtheria, even by intimate contact, develop the disease; likewise not a few persons carry in their upper air-passages diphtheria bacilli possessing a high degree of virulence, as shown by the guinea-pig inoculation test or by the infection of other persons with whom they come in contact, and yet show no clinical evidences of diphtheria. Occasionally individuals possessing this high degree of natural immunity to diphtheria may show in the tissues of the throat localized areas of superficial necrosis produced by virulent diphtheria bacilli, but escape the effects of toxemia and the production of the typical lesions of the disease.

Doubtless various local factors of resistance of the tissues of the nose and throat afford some degree of protection, but it is now known that the chief factor of defense is the presence of natural antitoxin in the blood.

Infants under one year of age are generally immune and known to escape diphtheria even when intimately exposed; in Philadelphia the incidence of the disease at this age is about 3 per cent., so that the immunity is not absolute, but at least 85 to 90 per cent. are known to possess antitoxic immunity which, presumably, is inherited from the mother. This immunity, however, gradually disappears, so that at the age of two years the majority of children are susceptible, and the highest percentage of cases of diphtheria develop in children from two to five years.

After twelve years of age immunity to diphtheria is gradually acquired by the majority of human beings so that at adult age from 80 to 90 per cent. are immune, the incidence of diphtheria in Philadelphia in individuals over eighteen years of age being in the neighborhood of 10 to 12 per cent. This immunity is probably due in part to the acquisition of a greater local resistance of the tissues in the nose and throat as compared with local resistance during childhood; also to the development of a natural antitoxin immunity probably the result of numerous minor infections with diphtheria bacilli capable of stimulating the production of antitoxin without producing well-defined clinical evidences of infection.

Aside from the purely local factors of resistance to diphtheric infection of mucous membranes, antitoxin in the blood constitutes the chief means of defense; von Behring has determined that the presence of approximately $1/30$ unit per cubic centimeter of blood-serum will protect a human being or guinea-pig against diphtheria. This antitoxin in the blood apparently neutralizes the toxin produced by virulent diphtheria bacilli and thereby prevents the necrosing influence of toxin upon the tissue cells with which the bacilli have come in contact; it also neutralizes the toxin absorbed in the lymphatic and vascular channels and thereby prevents toxemia and constitutional reactions.

Antitoxin, however, is not directly inimical to the diphtheria bacillus, but only to its exogenous poison; for this reason it does not directly destroy diphtheria bacilli and the latter may be present in the tonsils or other tissues with relatively large amounts of antitoxin in blood. The bacilli are probably largely destroyed by leukocytes, but antitoxin facilitates leukocytic de-

struction by neutralization of the toxins; in this manner the administration of horse-serum antitoxin is accompanied by gradual disappearance of the necrotic exudates and bacilli.

Tests for Antitoxin Immunity to Diphtheria.—For practical purposes tests for antitoxin in the blood-serum have proved satisfactory for detecting immunity to diphtheria. Schick¹ has discovered that if human beings are injected *intracutaneously* with one-fiftieth the minimal lethal dose of toxin for guinea-pigs and no reaction follows, sufficient antitoxin is present in the blood to confer immunity. This Schick test has been employed extensively during the past seven years, and *when properly conducted* has demonstrated its reliability and practical value.

In addition to this direct or clinical test for antitoxic immunity, it is possible to determine the presence or absence of antitoxin in the blood-serum by mixing serum and toxin together and after a suitable interval injecting a portion of the mixture into guinea-pigs subcutaneously or intracutaneously to determine if the toxin has been neutralized. Under proper technical conditions the dose of toxin is such as will produce necrosis at the site of injection unless neutralized and only diphtheria antitoxin in the serum being tested will effect neutralization. Kellogg² has recently advocated this test for practical use in place of the Schick test, and a description of the technic is given in Chapter XIII.

The Toxin for the Schick Test.—The proper preparation of the toxin is one of the most troublesome features of the Schick test. The actual amount to be injected *intracutaneously* may vary from about one-thirtieth to one-fiftieth the minimal lethal dose for 8- to 10-ounce guinea-pigs; my own preference is one-fortieth the minimal lethal dose.³ These amounts of undiluted toxin are too small in bulk for injection, so that it is necessary to dilute with sterile saline solution. Park and Zingher dilute with sufficient saline to make the dose injected 0.2 c.c.; Schick uses 0.1 c.c., and the writer has always preferred this amount as being less painful, less likely to produce trauma, and just as accurate.

The toxin must be potent and active and the amount injected neither too large (which may produce severe local reactions with necrosis) or too small (which may yield falsely negative reactions). The diluted toxin does not keep well and deteriorates within forty-eight to seventy-two hours; for this reason the toxin must be dispensed undiluted and diluted with saline solution just before injection. This complicates the practical application of the test and Zingher⁴ has recently discovered that the toxin dispensed for the test may deteriorate and become unsatisfactory. The outfit designed by Zingher⁵ and employed by the New York Department of Health is to be particularly recommended for conducting a small number of tests; for a large number of tests a vial of the undiluted bulk toxin should be used to be diluted by the physician according to the given directions. This insures against deterioration of toxin, greater accuracy in dilution is obtainable and the expense is only slightly greater; for these reasons the bulk toxin is preferable when a number of tests are to be made in hospitals and other institutions.

Zingher⁶ has suggested that the outfit of diphtheria toxin for the Schick test shall contain the following maximum and minimum contents of diphtheria toxin in order to allow for some deterioration and provide a sufficient amount:

¹ Münch. med. Wchn., 1913, lx, 2608.

² Jour. Amer. Med. Assoc., 1922, 78, 1782.

³ Amer. Jour. Dis. Child., 1915, 9, 189.

⁴ Jour. Amer. Med. Assoc., 1920, 75, 1333.

⁵ Ibid., 1915, 65, 329.

⁶ Ibid., 1922, 78, 490.

"1. If the directions on the outfit call for 0.2 c.c. as the dose for the Schick test, the toxin content shall be no more than 1.5 M.L.D. and no less than 1.25 M.L.D. to each 10 c.c. of diluent; 0.2 c.c. of the diluted toxin shall, therefore, represent no more than 1/33 M.L.D. and no less than 1/40 M.L.D. for a 250-gram guinea-pig.

"2. If the directions on the outfit call for 0.1 c.c. as the dose for the Schick test, the toxin content shall be no more than 2.5 M.L.D. and no less than 2 M.L.D. to each 10 c.c. of diluent; 0.1 c.c. of the diluted toxin shall, therefore, represent no more than 1/40 M.L.D. and no less than 1/50 M.L.D. for a 250-gram guinea-pig."

The M.L.D. or minimal lethal dose of toxin as defined by the Hygienic Laboratory, is the smallest amount of toxin that will kill the average 8- to 10-ounce guinea-pig in less than four days. This varies with individual animals, some dying on the third, others on the fourth day. The technic is described in Chapter XIII.

After the M.L.D. of a well ripened toxin has been accurately determined dilutions for the Schick test are made by first diluting the toxin so that each cubic centimeter contains 10 M.L.D. From this primary dilution the final dilution is prepared in such manner that each 0.1 c.c. (the amount injected intracutaneously in conducting the test) shall contain 1/40 M.L.D. The following table, slightly modified after that prepared by Zingher, summarizes the method:

METHOD OF DILUTION OF TOXIN FOR SCHICK TEST

M.L.D. of toxin (c.c.).	PRIMARY SCHICK DILUTION.			Number M.L.D. in each c.c. of P.S.D.	FINAL SCHICK DILUTION.		
	Amount of undiluted toxin, c.c.	Amount of salt solution, c.c.	Total primary dilution, c.c.		Amount of primary Schick, c.c.	Amount of salt solution, c.c.	Total final dilution, c.c.
1/150	1.0	14.0	15.0	10	1.0	39.0	40.0
1/125	1.0	11.5	12.5	10	1.0	39.0	40.0
1/100	1.0	9.0	10.0	10	1.0	39.0	40.0
1/90	1.0	8.0	9.0	10	1.0	39.0	40.0
1/75	1.0	6.5	7.5	10	1.0	39.0	40.0
1/60	1.0	5.4	6.0	10	1.0	39.0	40.0
1/50	1.0	4.0	5.0	10	1.0	39.0	40.0

The Control Fluid for the Schick Test.—In conducting Schick tests on individuals over six years of age it is advisable to include a control fluid in order to detect and properly interpret pseudo or falsely positive reactions and combined pseudo and true positive reactions. These pseudoreactions are produced in a large degree by the autolyzed protein of the diphtheria bacillus present in the culture broth and not removable by filtration; they will be described in more detail a little later on in this chapter.

For the purpose of checking these reactions Zingher¹ recommends the use of the same toxin as employed for the Schick test, but heated to 75° C. for ten minutes which destroys all of the toxin and only a slight amount of the autolyzed protein. After heating the toxin the primary dilution is made as described for the unheated toxin. The final dilution is then prepared by diluting 1.25 c.c. of the primary dilution with 39 c.c. of saline solution which gives a total of 39.25 c.c. of which the dose is 0.1 c.c. carrying a trifle excess of the heated toxin.

¹ Amer. Jour. Dis. Child., 1916, 11, 269.

Method of Conducting the Schick Test.—As previously stated, the *diluted toxin is injected intracutaneously and not subcutaneously*. The dose is either 0.1 or 0.2 c.c. according to the dilution which is always stated in the directions accompanying the outfit. Dr. Moshage and myself have tried a cutaneous test by applying undiluted toxin to an abrasion of the skin as in the technic of the cutaneous tuberculin test; reactions occurred after twenty-four and forty-eight hours, but these were seldom as definite and clear cut as the Schick test.

A good syringe and a fine needle are absolutely indispensable. The writer uses and can strongly recommend the 1 c.c. Record syringe divided in tenths and equipped with a tightly fitting gage No. 26 needle. The tuberculin syringe is also useful; it is essential that the syringe work properly without leakage so that an accurate and measured amount may be injected.

The toxin is injected into the skin of the right arm either below the elbow or about the insertion of the deltoid muscle; the control fluid may be injected in the same location on the left arm, but this injection may be omitted in children less than six years of age.

The skin should be cleansed with alcohol, picked up between the thumb and forefinger of the left hand, the needle inserted very superficially with the eye upward, so that the operator may be sure that the needle is introduced far enough and the injection given (Fig. 157). The injection is accompanied by a slight stinging pain and leaves a raised anemic spot (Fig. 158), showing the pits of the hair follicles. If this elevated area is not produced the injection has been too deep and should be repeated.

If one syringe is used it is better to inject the control fluid first and then the toxin, in order to make sure that none of the latter is carried into the control injection.

If more than one test is to be given, the same needle may be used for all, being wiped off with alcohol or 1 per cent. iodine solution between each injection. With proper assistance it is possible to give several hundred tests in an hour—much less time is required than for cowpox vaccination.

A dressing is not required; the needle puncture is so small that infection does not occur. The writer has never observed or heard of infection due to a properly conducted Schick test.

The readings may be made twenty-four hours later, if necessary, in order to determine whether or not antitoxin is required. If time permits, it is better, however, to wait forty-eight hours, which permits very slight traumatic reactions to subside. When the tests are conducted in conjunction with T-A immunization in which there is no necessity for haste in reading the reactions, it is better to make a final reading on the fourth or fifth day. With this interval there is no danger of missing the positive reactions; they are likely to be more clearly defined. Furthermore, the pseudoreactions will have faded to a large degree and the occasional pseudo and true combined reactions will be more clearly seen and differentiated from the negative pseudoreactions.

The Negative Schick Reaction and Its Significance.—In a negative reaction there is no redness and no infiltration because the injected toxin has been neutralized by the antitoxin in the blood. If the toxin was potent and the injection properly given, a negative reaction indicates that the individual has at least $1/30$ unit of antitoxin per cubic centimeter of the blood-serum which, according to von Behring and Schick and amply confirmed by others, is sufficient to confer immunity against diphtheria. In individuals over two years of age the immunity is permanent, that is, the antitoxin persists in the blood and subsequent Schick tests



FIG. 165.—THE SCHICK TEST FOR IMMUNITY IN DIPHTHERIA

A well-marked reaction thirty-six hours after the intracutaneous injection of one-fortieth the minimum lethal dose of a diphtheria toxin diluted to 0.1 c.c. The patient's blood contained no antitoxin. The brownish erythematous area with edematous infiltration of the subcutaneous tissues are characteristic of the reaction. (Reprinted from the *Amer. Jour. Dis. Children.*)

continue to yield negative reactions. *In children under two years, however, the immunity (negative reaction) may be only temporary because the inherited antitoxin may gradually disappear*; for this reason the Schick test is only of limited value in children under two years of age and may be omitted altogether.

The Positive Schick Reaction and Its Significance.—When the blood contains no antitoxin or less than one 1/20 to 1/30 unit per cubic centimeter, the injected toxin is not neutralized and acts as an irritant to the skin. In twenty-four hours there is a circumscribed area of hyperemia about the site of injection varying in size from a quarter to a silver dollar and accompanied by distinct edema (Fig. 165). This reaction reaches its height in three to four days and then gradually disappears leaving a definitely circumscribed scaling area of brownish pigmentation (Fig. 166) which persists for three to six weeks.

Severe reactions do not occur unless an error has been made and too much toxin injected, in which case the reaction may be quite large and accompanied by some vesiculation. When properly conducted there is no constitutional reaction and no tenderness of the axillary glands.

A *positive reaction indicates susceptibility to diphtheria*, although, of course, by reason of other factors, not all Schick positive individuals contract diphtheria when exposed. A positive reaction in children does not necessarily mean that the individual will be susceptible for life; it would appear that many acquire natural antitoxic immunity during later years, probably by reason of numerous minor infections stimulating the production of antitoxin.

The Pseudopositive Reaction and Its Significance.—Pseudopositive reactions, first described by Park, Zingher, and Serota,¹ are especially apt to occur in individuals over five years of age and may be present in as high as 25 per cent. of school children and adults.

Park and his associates have explained these reactions on the basis of local anaphylactic reactions to the protein of autolyzed diphtheria bacilli in the broth. Skin tests conducted by Moshage and the writer² with suspensions of detoxicated diphtheria bacilli (*diphtherin*), have yielded positive reactions of a similar kind, indicating that many individuals acquire anaphylactic sensitization to the diphtheria proteins; these reactions, however, bear no relation to immunity to diphtheria.³ In addition to this principal cause of pseudoreactions, I am of the opinion that trauma and non-specific enzyme reactions to the proteins of the broth independent of those contributed by the diphtheria bacilli, may aid in the production of these reactions.⁴

The pseudopositive reaction can be interpreted occasionally with fair accuracy by the trained observer from the true positive reaction; but there is usually some doubt as to the absolute certainty of such a reading unless the control injection of heated toxin has been given on the other arm for comparison. The pseudopositive reaction develops earlier, within six to eighteen hours, reaches its height in thirty-six to forty-eight hours, and is characterized by a central area of dusky redness about the size of a dime, with a characteristic secondary palish areola that shades off into the surrounding skin, and slight or no edema (Fig. 167). After fading it leaves none, or, but very slight pigmentation and scaling of the skin.

Occasionally and especially among adults, a *combined true and pseudo-*

¹ Arch. Pediat., 1914, 31, 481.

² Proc. Soc. Exper. Biol. and Med., 1916, 13, 89; Amer. Jour. Dis. Child., 1916, 12, 316.

³ Jour. Immunology, 1916, 1, 334.

⁴ Jour. Amer. Med. Assoc., 1915, 65, 144.

positive reaction may be seen. The central area of brownish redness is larger and accompanied by *distinct edema*; this is surrounded by the same palish erythema as seen in the pseudoreaction (Fig. 168). Definite brownish discoloration and scaling of the skin follows as in the true reaction. If heated toxin has been injected as a control, a typical pseudoreaction will be observed and the development of these control reactions indicates that special caution is necessary in reading and interpreting the reactions excited by toxin. When in doubt, it is better to regard the toxin reaction as a true positive and err if necessary on this side.

The Pseudoreaction in Relation to Toxin—Antitoxin Immunization; the Necessity of a Control.—Zingher¹ has recently emphasized that children yielding pseudo-Schick reactions are most likely to develop pronounced local and constitutional symptoms after injections of toxin—antitoxin. This is to be expected when it is remembered that the pseudoreaction is principally due to sensitization to the proteins of the diphtheria bacillus contained in the toxin broth. For this reason the control injection is always advisable, indeed, is almost a necessity, in Shick tests on individuals over five years of age and especially if T-A immunization is to be given; by means of the control injection the pseudoreactions are readily detected and given special attention in active immunization. The control injection also aids in the interpretation of combined pseudo- and true Schick reactions; in such individuals the first dose of T-A should be about one-half that ordinarily given (0.5 c.c.) in order to reduce the degree of reaction that may follow.

The Percentage of Positive Schick Reactions and the Influence of Age, Race, and Inheritance.—The percentage of positive reactions at different ages reported by different investigators and even by the same investigator at different times, have varied to a considerable degree. Doubtless these variations have been largely due to defective toxin (too much, too little, or inactive), errors in injection and interpretation of results; also according to locality (urban or country districts), race and social condition, and general health. For example, Zingher² has observed in New York City a much higher percentage of positive Schick reactions (as high as 75 per cent.) among school children from the homes of the more well-to-do than those from the homes of the poorer classes living in closely crowded neighborhoods (as low as 16 to 20 per cent.), where the chances of contact infection and the development of natural immunity are greater. An unusually low proportion of positive reactions occurred among the children of Italian parents.

An exception to this general rule was noted among colored children, the percentage of positive reactions being high despite the fact that the majority lived in the congested districts. Wright,³ however, observed about the same percentage of positive reactions among adult negroes as white adults.

Park, Zingher, and Serota also found a marked tendency for all children of the same family to show a similar Schick reaction, whether it was positive or negative. Where variations occurred, the younger children as a rule gave a positive reaction and the older children a negative; the reverse condition was very rare, except in families with young infants, who often gave a temporary negative reaction due to inherited immunity.

Bearing in mind these modifying factors it is almost useless to attempt expressing the percentage of positive Schick reactions at different ages; 52,000 tests conducted by Zingher among the school children of New York varying in age from six to fifteen years, or thereabouts, have shown positive

¹ Jour. Amer. Med. Assoc., 1922, 78, 1945.

² Ibid., 1921, 77, 835.

³ Jour. Infect. Dis., 1917, 21, 265.



FIG. 166.—A FADING SCHICK REACTION SHOWING BROWNISH DISCOLORATION AND FINE DESQUAMATION.



FIG. 167.—A PSEUDOPOSITIVE SCHICK REACTION.



FIG. 168.—A COMBINED TRUE AND PSEUDOPOSITIVE SCHICK REACTION.

reactions varying from 13.6 to 67 per cent. and pseudoreactions varying from 3.9 to 26 per cent. Figures, however, will indicate in a general way the influence of age and may be given as follows:

	Per cent. positive.
One to six months.....	15
Six to twelve months.....	50
One to two years.....	55
Two to four years.....	65
Four to six years.....	40
Six to eight years.....	35
Eight to twenty years.....	25
Twenty to forty years.....	18
Over forty years.....	12

The Influence of Disease Upon the Schick Reaction.—In 1915 Moshage and the writer¹ called attention to the higher percentage of positive Schick reactions occurring among persons with scarlet fever. Zingher² observed that the reactions were twice as frequent in this disease, slightly more frequent during measles, and nearly three times as high during poliomyelitis. Lereboullet,³ however, did not find that measles influenced the reactions.

The Relation of the Schick Test to Diphtheria Carriers and to the Diagnosis of Diphtheria.—There is no relation between the percentage of carriers of diphtheria bacilli and the Schick reaction. An individual may carry virulent bacilli in the nose and throat without evidences of diphtheria and yield a negative Schick reaction, because of the presence of sufficient antitoxin in the blood to protect against that produced by the bacilli and to neutralize that injected into the skin. As previously mentioned, however, Park has observed that virulent diphtheria bacilli may produce some inflammatory changes in the tonsils with superficial necrosis in Schick negative individuals; this indicates that the bacilli and their toxins may produce some tissue changes when so superficially located as to be beyond the neutralizing influence of antitoxin in the blood.

Individuals may carry diphtheria bacilli and yield positive Schick reactions. In the majority of such cases the bacilli are found non-virulent for guinea-pigs. For this reason the Schick test is of some aid in the management of cases showing diphtheria or diphtheroid bacilli in nasal and aural discharges; if the Schick reaction is positive it indicates that the bacilli are very probably non-virulent, for the positive skin reaction indicates the absence of antitoxic immunity in which case the patient would probably show clinical evidences of diphtheria if the bacilli were virulent. I have observed, however, positive Schick reactions among diphtheria convalescents harboring virulent bacilli; doubtless there are other local tissue and immunity principles that may protect an individual against virulent diphtheria bacilli even though lacking in antitoxic immunity.

The Schick test, therefore, is not a substitute for the culture method in the diagnosis of diphtheria and the detection of carriers. It aids, however, in the management of carriers as discussed above. The test is not an index of the virulence of bacilli recovered in cultures; the guinea-pig inoculation test is the only reliable one for this purpose.

The Practical Value of the Schick Test.—A large number of investigators have found the Schick test safe and a reliable and simple means for determining the presence or absence of immunity to diphtheria. Accidents have happened, severe reactions having been produced by the injection of too

¹ Amer. Jour. Dis. Child., 1915, 9, 189.

² Amer. Jour. Dis. Child., 1917, 13, 247.

³ Bull. d. l. Soc. Méd. d. Hôp., 1921, 45, 1210.

much toxin; likewise a few have claimed that diphtheria developed among those yielding negative reactions, but the great bulk of evidence is favorable to the test and indicates unmistakably that when *properly conducted* the test is without danger and of much value. A large literature has accumulated among which may be mentioned, in addition to those previously referred to, the reports of Weaver and Maher,¹ Bundesen,² Moody,³ Graef and Ginsberg,⁴ Linenthall and Rubine,⁵ von Gröer and Kassowitz,⁶ Lilly,⁷ Leet,⁸ Koplik and Unger,⁹ Moffet,¹⁰ Conrad,¹¹ de Elizaldi,¹² Cowie,¹³ White,¹⁴ Mulsow,¹⁵ Blum,¹⁶ and others.

Unfortunately, the practical application of the test is hampered by the chances of using defective toxin and the necessary procedures for diluting and injecting it; also by the occurrence of pseudoreactions, but these difficulties soon disappear with a little experience and especially when bulk toxin can be used as required for a large number of tests in schools, hospitals, and other institutions.

The test has its greatest value as a means for detecting those lacking in antitoxic immunity and requiring immediate protection by an injection of antitoxin when exposed to diphtheria or, in the absence of acute exposure, slower but more lasting immunization by toxin-antitoxin mixtures (see Chapter XLI). By means of this test a large number of individuals may be spared the discomfort and useless sensitization caused by injections of horse-serum antitoxin and it has given great impetus to active immunization with T-A mixtures.

The *influence of age upon the reaction* is to be remembered, that is, that a child under two or three years of age may react negatively and later positively, when its inherited immunity has disappeared. For this reason young children should be retested at intervals to detect susceptibility should such develop. After four or five years of age the reaction is greatly stabilized, that is, continues to yield negative reactions probably for life, or positive reactions, unless the individual is immunized.

It is not necessary, however, to use the test on children under five years in relation to toxin-antitoxin immunization; the majority give positive reactions anyhow, and the Schick test may be omitted in this group and T-A given to all children between six months and five years. After six years of age, however, the Schick test and its control should be conducted.

Naturally the test has its greatest usefulness under conditions where the spread of diphtheria is facilitated, as in the wards for children in hospitals, in institutions for children and adults as well, among nurses, physicians, and others brought in contact with diphtheria or diphtheria carriers. Cooke¹⁷ and others have shown the value of the test among nurses and immuni-

¹ Jour. Infect. Dis., 1915, 16, 292.

² Jour. Amer. Med. Assoc., 1915, 64, 1203.

³ Jour. Amer. Med. Assoc., 1915, 64, 1206.

⁴ Jour. Amer. Med. Assoc., 1915, 64, 1205.

⁵ Boston Med. and Surg. Jour., September 16, 1915.

⁶ Ztschr. f. Immunitätsf., 1914, 22, 404; *ibid.*, 1914, 23, 108.

⁷ Boston Med. and Surg. Jour., 1920, clxxxii, 110.

⁸ Lancet, January 24, 1920.

⁹ Jour. Amer. Med. Assoc., 1916, 66, 1195.

¹⁰ Jour. Amer. Med. Assoc., 1915, 64, 1203.

¹¹ Jour. Amer. Med. Assoc., 1915, 65, 1010.

¹² Prensa Méd., 1919, 5, 293.

¹³ Amer. Jour. Dis. Child., 1916, 12, 266.

¹⁴ Boston Med. and Surg. Jour., 1921, 184, 246.

¹⁵ Jour. Amer. Med. Assoc., 1921, 77, 1254.

¹⁶ Amer. Jour. Dis. Child., 1920, 20, 22.

¹⁷ Amer. Jour. Dis. Child., 1922, 23, 496.

zation of susceptibles by injection of toxin-antitoxin mixtures. In the writer's opinion the Schick test should be conducted routinely under these circumstances and the positive reactors either actively immunized with toxin-antitoxin mixtures or passively with antitoxin, when intimately exposed.

In private practice physicians will do well to acquaint parents with the advantages of the Schick test and active immunization and to encourage their use. By grouping individuals in private practice a number of tests may be done in one day, thereby minimizing the work involved.

It is very much hoped that state and city Departments of Health will facilitate the application of the Schick test and active immunization by providing suitable outfits and even trained physicians for conducting the tests, especially in institutions and schools. The work being carried out in the schools of New York City consisting in conducting the Schick test and actively immunizing the positive reactors, should be closely followed, as it bids fair to prove a useful and practical means for really reducing the incidence of diphtheria.

CHAPTER XXXIV

PRINCIPLES OF ACTIVE IMMUNIZATION—VACCINES IN THE PROPHYLAXIS AND TREATMENT OF DISEASE

WHILE the importance of natural immunity must not be underrated in the protection it gives us after bacterial invasion has occurred, this immunity is, however, usually relative and seldom absolute, and may afford insufficient protection if the invading bacteria are numerous, or particularly virulent, or if the natural resistance of the organism is weakened by fatigue, disease, or injury.

Active Immunization.—Usually the best and most lasting immunity is that actively acquired, in which our own body cells are stimulated or trained, as it were, to produce specific antibodies against the offensive forces of a particular bacterium or other pathogenic agent. A well-marked and lasting degree of this form of immunity usually follows recovery from many of the acute infections, particularly the acute exanthemata, such as smallpox, scarlatina, measles, typhoid fever, typhus fever, etc. In other infections, such as erysipelas, gonorrhea, and pneumonia, the immunity is less complete, of short duration, or apparently absent, and, indeed, a state of hypersusceptibility to infection may actually follow.

It is very important, in this connection, to remember that the degree of immunity is not necessarily in proportion to the severity of the disease; thus a mild infection may be followed by the much-desired immunity, and while in general there is not considerable *protection without infection*, the latter does not necessarily imply that a virulent infection, or even the actual disease itself, is present, for discoveries have shown that an active immunity may be acquired by inoculation with the antigen so modified or attenuated that it can stimulate the production of specific antibodies without producing the disease or otherwise greatly disturbing the health of the individual.

Historic.—The facts here detailed are well illustrated in the history of vaccination in smallpox and the development of vaccine therapy in general. Hundreds of years ago the people of the eastern countries were accustomed to expose their children to a mild case of smallpox in order that a similar mild infection might be acquired and a lasting immunity thus secured with the least danger to life. This practice, however, was not without risk, as the mild disease not infrequently became a virulent one. Later the dose of infectious agent was decreased by applying the virus to a small abrasion on the skin, and the resulting mild but genuine attack of smallpox usually conferred the much-desired immunity. But here again the severity of the disease was not under control, as the virus occasionally assumed increased virulence and induced severer infections than were desirable. Finally, Edward Jenner observed and showed experimentally (1796) that when cowpox virus is inoculated into the human being, a trivial infection, since called “vaccinia,” is induced, and that this is followed by an absolute or nearly absolute immunity of many years’ duration against smallpox. In other words, the virus, in its passage through the cow, becomes so modified that it can no longer produce smallpox, but is still able to stimulate the production of the specific antibodies against this disease. Jenner worked so hard to establish the truth of this finding that he had little time to devote to the mechanism involved in the process.

In other words, the work of Jenner was largely empirical, and the explanation was not forthcoming until many years later, when Pasteur laid the basis of scientific immunization by discovering that light, high and low temperature, and exposure could so reduce the virulence of a micro-organism that while its injection into an animal was practically without danger or ill effect, it could still stimulate the protective mechanism of the host and induce a high degree of immunity.

That this could be done was a fact discovered accidentally by Pasteur in 1879 while working with the organism of chicken cholera. After an absence from home he found on examining his cultures on his return that they had become innocuous—that hens could bear without any ill effect inoculation of what would formerly have been a lethal dose. The prolonged cultivation of the micro-organism had caused its attenuation and Pasteur immediately grasped the far-reaching importance of this discovery. He conjectured that it might be possible to produce a mild and modified form of chicken cholera with a vaccine of the attenuated micro-organism which would afford protection to the fowl against the severe form of the disease. This proved to be the case, and established the possibility of so modifying or attenuating the virulence of a virus or germ that, while its administration is not followed by the actual disease, it is capable of so stimulating the body cells that the specific antibodies are produced. This discovery formed the basis of prophylactic immunization or bacterin therapy in general.

Following this discovery much work was done, for it appeared that the question of prevention of any bacterial disease simply depended upon whether the bacterium could be cultivated and so modified or attenuated that while its injection would not be followed by disease or other harmful effects, it would be capable of causing the production of specific antibodies.

Naturally, most of the earlier work was done with the infections of the lower animals, and, consequently, most discoveries were directly beneficial to them. Pasteur soon devised a method of attenuating anthrax bacilli by exposing them to certain temperatures for varying lengths of time, so that a vaccine could be prepared that has proved of great value. Later the same observer discovered a method of attenuating the virus of hydrophobia by a process of drying, and devised a practical method of prophylactic immunization against this disease. In addition to these his vaccines against swine erysipelas, symptomatic anthrax, and rinderpest have become well known.

The knowledge gained from a study of the diseases of the lower animals and the aid given them has been applied to human medicine with considerable benefit not only in prophylactic immunization but also in therapeutics (bacterin therapy). The latter application is a more recent discovery, for which we are mainly indebted to the researches of Wright, Leishman, Douglas, and their colleagues.

Nomenclature.—The word *vaccine* is from the Latin *vacca* (a cow). Cowpox was called “vaccinia,” or the cow disease, and Jenner designated protective inoculation against smallpox with cowpox virus as *vaccination*. With true courtesy Pasteur adhered to Jenner’s nomenclature and applied the term *vaccine* to emulsions of dead or attenuated bacteria. This is unfortunate and tends to create confusion, as the term *vaccine* is inseparably associated with cowpox virus or lymph. The term *bacterial vaccine* has become widely known, and is used to designate bacterial suspensions prepared for purposes of immunization. There is no essential difference, however, between cowpox vaccine, which contains the modified germ or virus

of smallpox in a diluent of lymph, and a bacterial vaccine containing the germ, modified by some physical or chemical agency in a diluent of saline solution or bouillon. It is, however, well to reserve the unqualified term "vaccine" for cowpox virus and to retain the designation "bacterial vaccine" for suspensions of attenuated or dead bacteria. More recently the term *bacterin* has been suggested and applied by S. Solis Cohen to the latter, but this would imply an extract of bacteria, as, *e. g.*, tuberculin, which is not always the case.

Some confusion likewise exists as regards the terms *serum* and *vaccine therapy*. *Serum therapy* is a process of *passive immunization* induced for either protective or curative purposes by the injection of the blood-serum of another animal that has been actively immunized by inoculation with bacterial toxins or the bacteria themselves, as, for instance, the injection of diphtheria or tetanus antitoxins. *Vaccine* or *bacterin therapy* is a process of active immunization brought about by the injection of the bacteria or their products directly into a patient. Bacterial vaccines that are simple emulsions of dead or attenuated bacteria are not, therefore, serums, and the indiscriminate use of the two terms is much to be regretted.

Methods of Preparing Vaccines.—It may be stated that, in general, the specific micro-organism or virus used in a vaccine should be modified as little as possible, or just sufficient to rob it of its disease-producing power. For example, typhoid bacterial vaccine is prepared by suspending the bacilli in salt solution and exposing them to just enough heat to modify them so that they can no longer multiply. The less modification, the better the vaccine. If the exposure is too prolonged or the temperature too high, the vaccinogenic power of the micro-organisms is likely to be reduced or destroyed. Therefore the nearer the vaccine approaches the fully viable virus or micro-organism, the more potent it will be. The proper preparation of a vaccine, therefore, is the first step to successful vaccine therapy.

Vaccination, using the term in its broadest sense, may be performed for prophylactic purposes and curative immunization in the following ways:

1. *The living micro-organism may be inoculated.* This is the ideal method, but for obvious reasons has not been generally used and is still in the experimental stage. It is based upon experimental observations made on the lower animals that an organism may be so introduced as to render it incapable of producing disease, but may, however, stimulate the production of specific protective antibodies. Evidence thus far indicates quite conclusively that the typhoid bacillus, for example, is unable to produce typhoid fever unless it is introduced into the gastro-intestinal tract, and the subcutaneous injection of living bacilli, modified only to a slight extent by artificial cultivation, is not followed by ill effects and produces a high grade of immunity. Rabies vaccine, for example, is sometimes prepared of the living virus greatly diluted with saline solution for the initial doses. The principle is a good one, *i. e.*, in a vaccine the micro-organism should be modified as little as possible. For obvious reasons, however, this method must be thoroughly tried out on susceptible lower animals before it is applied to human beings.

2. *By inoculation with a modified virus or with micro-organisms attenuated or modified according to various methods.*

- (a) By passing the virus through a lower animal, as the passage of smallpox through the heifer when the virus is incapable of producing smallpox, although vaccinia confers a specific immunity against smallpox. A vaccine for swine erysipelas is prepared in the same manner (Pasteur) by passing the bacillus through the rabbit several times, which increases its virulence for the rabbit, but decreases it for swine.

(b) By exposing suspensions of micro-organisms to heat. They are usually grown on a suitable solid medium, suspended in salt solution, and exposed to a temperature at or just above their thermal death-point for just sufficient time to kill or attenuate them in so far that they cannot multiply. The same result may be secured by longer exposure to a lower temperature. *To secure a potent vaccine the principle of minimum exposure at the minimum temperature should be observed*, the question of viability being controlled by culturing the vaccine. Most bacterial vaccines are prepared in this manner. Usually an exposure of 53° to 60° C. for from one-half to one hour is sufficient, and only exceptionally are these limits exceeded.

(c) By exposing the micro-organism to air and light. The first bacterial vaccine (chicken cholera) was accidentally prepared by Pasteur in this manner.

(d) By desiccating or drying the virus. This is one of the methods of vaccination in rabies, as the virus contained in the spinal cord of rabbits is dried for varying lengths of time, emulsified, and injected. The longer the period of drying, the greater the attenuation, and in this manner the strength of the vaccine and the progress of immunization are under control.

(e) By exposing the micro-organism to a high temperature for varying lengths of time. Anthrax vaccine, for the immunization of lower animals, is prepared in several strengths by exposing suspensions of the bacilli to 42° C. for varying periods of time.

(f) By exposing the micro-organisms or their products to certain chemical germicides, as in the preparation of anthrax vaccine by Roux, diphtheria and tetanus toxins (Behring), in some preparations of tuberculin, and in the preparation of rabies vaccine and ordinary bacterial vaccines by sterilization with phenol, etc.

3. *By inoculating with bacterial constituents, as the soluble toxins, bacterial extracts, and products of bacterial autolysis*, as in the preparation of Koch's tuberculin T. R., Koch's old tuberculin, mallein, diphtheria and tetanus toxins, Rosenow's pneumococcus vaccine, Gay's typhoidin, etc.

In Chapter XII a method is given for preparing bacterial vaccines, of which typhoid vaccine is a type. Special methods of preparing certain bacterial vaccines and other vaccines, such as cowpox virus and rabies vaccines, are given in this chapter.

The Relation of the Method of Preparation of Vaccine to Immunizing Activity; Living Versus Killed Vaccines.—Barring accidents, the employment of a living vaccine would appear to be the most certain way of calling forth a maximum output of antibodies; this is indicated by the remarkable and unparalleled success of cowpox vaccination as ordinarily conducted with living virus. There is at present no satisfactory explanation for this except that heat-labile substances destroyed in the ordinary preparation of bacterial vaccines have antigenic properties (Smith). Living vaccines are also capable of penetrating into deeper tissues, whereas dead vaccines may remain where they are deposited. Similarly living viruses are capable of exerting a continuous action and of delivering an infinite number of blows, whereas the injection of a dead virus produces an interrupted action and deals but a single blow. The actual dangers of using a living vaccine, as the possibility of it being too virulent and thus producing disease, or some of the symptoms as paralysis during the course of antirabic vaccination, or of regaining virulence or producing chronic "carriers" preclude their general employment in human practice.

To prevent these accidents ordinary bacterial vaccines are generally heated at 56° to 60° C. for one hour for purposes of sterilization, but this

reduces vaccinogenic activity. Crandon,¹ in a summary of the literature on the use of killed and living vaccines, concludes that unheated vaccines have proved superior. In order to avoid the destructive influence of heat Casselman² and more recently Kisskalt³ have advocated the use of phenol-killed vaccines, which had previously been employed for dysentery vaccine by Gay; Vincent⁴ has employed ether, and others galactose, sodium fluorid, iodine, and other chemical substances. In the preparation of antibacterial sera, as antimeningococcus and antipneumococcus serum, killed cultures produce an antibody response, but more recently it has been shown that smaller doses of living micro-organisms produce a quicker and more pronounced reaction, and if this experience can be borne out in active immunization in man, it will appear as support for the contention that the superior vaccines are those produced with the least possible change in the bacterial protoplasm. It cannot be questioned, however, that when vaccines are prepared in such manner that the bacterial protein undoubtedly undergoes some more or less profound chemical change, as in the method of preparation advised by Löffler, which consists in heating the cultures to 120° to 150° C., followed by drying or pulverizing, such vaccines possess some degree of antigenic activity capable of eliciting both specific and non-specific responses. Sommerville⁵ likewise found that heating vaccines at high temperatures, as boiling for fifteen minutes, did not reduce antigenic activity, but Taylor⁶ observed that the activity of these was slightly less than with vaccines heated at 60° C. Gay and Claypool,⁷ in an extensive series of experiments, have shown that typhoid bacilli flocculated by alcohol and dried at ordinary temperatures followed by grinding to a fine powder retain antigenic activity; Brown⁸ has observed similar effects.

In a study of the immunizing activity of typhoid vaccines prepared after various methods Perry and myself⁹ found that living bacilli were most active followed by chemically killed vaccines; heat-killed vaccines proved least antigenic for rabbits.

The subject is one of considerable importance in relation to active immunization both for prophylactic and therapeutic purposes, and shows that the method of preparing vaccines is one deserving of more attention. Unquestionably the highest degree of active immunization is engendered by living micro-organisms as indicated by the lasting immunity following one attack of many of the acute infectious diseases and the success of cowpox vaccination. On the other hand, the products of bacterial activity, as, for example, diphtheria toxin, and dead and thoroughly disrupted bacterial cells, possess antigenic activity and being free of certain toxic elements may actually prove more antigenic than living micro-organisms as shown by the success of vaccine therapy in acute bacterial infections.

Variation in the Antigenic Activity of Different Microparasites.—While the method of preparation of vaccine unquestionably exerts some influence upon immunizing capacity, it would appear that the immunity response to vaccines of different microparasites likewise varies within wide limits. For example, a vaccine of the typhoid bacillus prepared by the usual method possesses a high degree of antigenic activity, whereas vaccines of *Treponema pallidum* prepared and administered in exactly the same manner are apparently followed by the production of only small amounts of demonstrable

¹ In Sanborn's Surgical After-treatment, Saunders Co., 1910, 763.

² Jour. Amer. Med. Assoc., 1915, 64, 328.

³ Deut. med. Wchn., 1915, 41, 393.

⁴ Compt. rend. Acad. des Sci., 1912, clv, 480.

⁵ Lancet, 1915, 2, 96.

⁶ Lancet, 1915, 2, 150.

⁷ Archiv. Int. Med., 1914, 14, 671.

⁸ Indian Jour. Med. Res., 1913, 1, 589.

⁹ Jour. Immunology, 1918, 3, 247.

antibodies in the blood and a practically negligible degree of actual immunity. In other words, vaccines of different microparasites prepared and administered in exactly the same manner stimulate widely different degrees of immunity, due either to a difference in the degree of antigenic activity of the microparasites or to a difference in the capacity for immunity response on the part of the body cells.

In a series of experiments by Miss Trist and myself rabbits were injected with fixed and constant amounts of vaccines of glanders, typhoid, tuberculosis, diphtheria, anthrax and subtilis bacilli, gonococci, meningococci, streptococci, pneumococci, and staphylococci; each vaccine contained the same number of bacteria per cubic centimeter, prepared in exactly the same manner and injected intravenously into rabbits in similar amounts per body weight. Agglutination and complement-fixation tests were conducted at regular intervals and marked variations in antibody response were observed. The vaccines of Gram-negative bacilli (glanders and typhoid) stimulated the production of more agglutinins and complement-fixing antibodies than the Gram-positive bacilli (tuberculosis and diphtheria) and the spore-forming bacilli (anthrax and subtilis) produced almost none of these. The Gram-negative cocci (gonococci and meningococci) were more antigenic than the Gram-positive cocci (pneumococci, streptococci, and staphylococci).

These results are to be interpreted, of course, only in relation to the production of agglutinins and complement-fixing antibodies inasmuch as opsonic determinations were not made, but it would appear that bacterial proteins vary considerably in vaccinogenic activity in the same animal when given in the same amounts per body weight, and that this bears an important relation to the question of success of prophylactic and therapeutic immunization.

Sensitized Vaccines.—Besredka and Metchnikoff¹ have suggested a plan of injecting a vaccine composed of living bacteria that have been immersed in their specific immune serum or, in other words, have been *sensitized*. They believe that such vaccines produce practically no negative phase, but only slight local and general reaction, and that the general response with antibody formation is facilitated. This principle is supported by the observations of Theobald Smith, who found that the experimental injection of a toxin-antitoxin mixture aids the dissemination of the toxin through the body quite generally, whereas the pure toxin is chiefly held at or near the place of injection. This diffusion tends to cause maximum antibody formation over an entire portion of the body by a relatively small amount of free or easily dissociated toxin in the toxin-antitoxin mixture. Smith inclines to the belief that a similar phenomenon of diffusion may occur with sensitized dead bacteria.

In addition, the specific immune serum may aid in the disintegration of the bacterial cell, either through the attachment of a bacteriolytic amboceptor that would tend to lyse the bacterium with a complement of the tissues, or through a preliminary action of opsonin which prepared the bacterium for ultimate destruction and liberation of antigenic principles.

Blandini,² in a careful study of the immunizing properties of seventeen typhoid vaccines carried out on guinea-pigs, found that the sensitized vaccine of Besredka³ was the most protective. Several investigators have found that sensitized vaccines engender the production of agglutinins rather

¹ Ann. de l'Inst. Pasteur, 1911, xxv, 193, 867; 1913, xxvii, 597, 607.

² Ann. d'igiene sperimentale, 1905, 15, 295.

³ Ann. de l'Inst. Pasteur, 1902, 16, 918; Compt. rend. Acad. d. Sci., 1902; Bull. Inst. Pasteur, 1910, 241.

poorly, but better bacteriolysins and complement-fixing antibodies than plain vaccines. Garbat and Meyer¹ have found that the sera of animals immunized with sensitized vaccines protects passively better than the sera of animals immunized with plain vaccines. Cecil² employed sensitized vaccines in a series of 47 cases and found that the reactions are somewhat milder, but that the immunity is not higher than that engendered by plain vaccines.

While Besredka originally advocated sensitized living vaccines, most vaccines of this kind are prepared of killed cultures sensitized by immune goat- or horse-serum. Gordon³ states that the cultures killed by phenol are as good as the former without the danger of infection. Wohl⁴ has advocated the use of autogenous vaccines killed by heat and sensitized by the patient's own serum.

Doubtless sensitized bacteria are more rapidly amenable to phagocytosis by reason of preliminary opsonification than plain vaccines, and this probably aids their quicker absorption from the subcutaneous tissues with less local reaction than follows the injection of plain vaccines. These advantages, however, are not marked, and the superiority of sensitized killed vaccines is more theoretic than practical on the basis of actual experience.

Mixed Vaccines.—Of great practical importance is the question of the immunizing activity of mixed vaccines, that is, vaccines composed of two or more different kinds of bacteria. Castellani⁵ in 1903 showed that on injecting an animal with two different organisms at the same time, agglutinins were produced for both, and that the amount of agglutinin for each was about the same as in those animals immunized with but one type of organism. Subsequently he stated that as many as six different kinds of bacteria might be combined in a single vaccine with the same result, but that if more than six were employed, a diminished amount of agglutinin for each type resulted. During the recent World War Castellani advocated the use of these mixed vaccines for prophylactic immunization, and especially mixtures of typhoid, paratyphoid, cholera, and dysentery bacilli.

Davison⁶ likewise found that immunization of rabbits with mixtures of typhoid and the paratyphoid bacilli yielded for each organism as much and usually a greater response of agglutinin production than the single vaccines. Noble and Thomas,⁷ however, found that the bacteriolysins engendered by the administration of mixtures of typhoid and paratyphoid bacilli were somewhat less than produced by vaccines of single strains.

Smith⁸ has reported a marked example of polyvalency in the production of "ferments" against bacterial antigens. Huntoon and Craig⁹ have likewise found that in the immunization of horses with pneumococci, streptococci, and influenza bacilli there is nothing in the immunity mechanism itself to preclude the possibility of a wide polyvalent antibody production.

These results indicate that antibody production may be engendered by mixtures of different kinds of bacteria and that under proper technical conditions of dosage, etc., the antibody response for each may be as great as when monovalent vaccines are employed. However, the immunity

¹ Ztschr. f. exper. Path., 1910, 8, 1.

² Amer. Jour. Med. Sci., 1918, 155, 781.

³ Lancet, 1913, 1796.

⁴ Amer. Jour. Med. Sci., 1916, 152, 262.

⁵ Ztschr. f. Hyg., 1902, 40, 1; Jour. Trop. Med., 1914, 17, 326.

⁶ Arch. Int. Med., 1918, 21, 437.

⁷ Proc. Soc. Exper. Biol. and Med., 1920, 17, 190.

⁸ Jour. Infect. Dis., 1915, 16, 313.

⁹ Jour. Immunology, 1921, 6, 235.

response to mixed vaccines may not be as great as when single vaccines are employed when the amounts of the former injected are sufficient to produce more marked reactions and consequent depreciation of the general physical condition. For this reason the number of different bacteria incorporated into a vaccine should be limited as far as possible.

Mixed vaccines for therapeutic immunization are commonly employed in the treatment of chronic infections of exposed mucous membranes and notably of the ear and respiratory tract. At the present time there is no practical means for determining which bacteria in cultures are pathogenic and thereby important from the standpoint of vaccine therapy, although some aid in this direction is given by skin tests and especially in allergic asthma. As a general rule and principle vaccines should be prepared of as few different bacteria as possible and only those incorporated into a vaccine in full dosage which are known or reasonably suspected to be pathogenic.

Autogenous versus Stock Bacterial Vaccines.—It may be stated in general that autogenous vaccines, *i. e.*, those prepared from the patient's own bacteria, should be used whenever possible, especially in the vaccine treatment of disease. To be successful, vaccine therapy demands that the bacteria be as little changed as possible. Before they are killed the bacteria should be endowed with as many of the potencies as possible with which they maintain themselves in the body. As these potencies do not remain unchanged during artificial life, as the loss of capsules, loss of virulence, etc., it is advisable to secure the organism causing the infection as quickly as possible and prepare a vaccine without undue delay.

Variants may occur among cultures of the same species, and the injection of one strain may not protect against another, as shown by Neufeld for the pneumococcus. In the use of an autogenous vaccine this risk of using an alien species or a different strain is reduced to a minimum.

In some cases the difficulty of securing and of identifying the infective agent may be so great that much time is lost in preparing autogenous vaccines, as, for instance, in gonorrheal and tuberculous infections, and in such cases it may be necessary to use a stock vaccine.

In protective immunization stock vaccines are used, as, for instance, in the preparation of typhoid vaccine. In certain instances, as in gonococcus and tuberculous infections, stock vaccines possess but slightly inferior therapeutic value as compared with autogenous vaccines, not to mention the delay and difficulty in cultivating and preparing autogenous vaccines.

Stock vaccines are generally required when vaccine therapy is employed in acute infections, as pneumonia, streptococcus cellulitis, and typhoid fever; at least they may be employed until the autogenous micro-organism can be recovered in culture and prepared in a vaccine.

As a general rule stock vaccines should be polyvalent in order to include strains that may be serologically distinct. While autogenous vaccines are to be preferred in the treatment of disease providing they are properly prepared, stock vaccines possess equal powers for non-specific immunization and serve useful purposes.

Specific Antigenic Activity of Vaccines.—The injection of dead bacteria in vaccines may cause the production of specific antibodies. The mechanism is similar to that involved during infection with the living micro-organism, and involves the first principles of immunity. The antigenic powers of a vaccine are probably always more or less inferior to the living antigen, as some principle may be lost during heating, drying, passage through animals, the action of germicides, etc.

Just what portion of the bacterial cells is mainly antigenic it is difficult

to determine, for it probably varies with different species. With a true toxin, as, for example, the diphtheria bacillus, the toxin constitutes the main principle, and causes the production of an antitoxin as its main antibody; with other bacteria, such as the typhoid bacillus, a soluble toxin and an endotoxin in combination with the protein of the bacterial cell are probably the main antigenic factors responsible for the formation of a bacteriolysin, opsonin, antitoxin, agglutinin, etc.

In brief, the antigenic principles of a micro-organism are mainly thermostable and fairly resistant substances, so that a bacillus may be so attenuated or altered that it cannot multiply or produce disease, and yet is capable, through the agency of substances that have escaped destruction, of causing the production of specific antibodies.

As was previously stated, vaccines may cause the production of different antibodies. As curative agents, however, it would appear that they are most efficacious in those infections in which phagocytosis is known to be chiefly concerned in the defense of the host, *e. g.*, in staphylococcus infections. As shown by Wright and Douglas, Neufeld and Rimpau, a bacterial vaccine facilitates phagocytosis, not so much qualitatively or quantitatively, as through the production of specific substances that act directly and primarily upon the bacteria and render them more vulnerable to phagocytosis (opsonin or bacteriotropin). Wright has advised a method of opsonic measurement, previously described, for measuring the immunity response, but, as will be understood, while the opsonin may be the chief antibody, it is seldom if ever the only one, so that the opsonic index is but one measure of defensive power.

According to Vaughan, a micro-organism is directly responsible for the production of a specific proteolytic ferment capable of causing the disintegration or destruction of the bacterial cell and its products. The ferment is the antibody, and is produced during an infection or by a vaccine in just the same manner as antibodies in general are produced. In other words, Vaughan regards antibodies as of the nature of proteolytic ferments; thus the protein of the micro-organism composing a vaccine produces a specific proteolytic ferment capable of overcoming its substratum when it meets the latter in the form of the invading micro-organism of an infection.

For example, the tissues affected may be unable to produce a sufficient quantity of the specific ferment necessary to overcome the infection. The injection of bacterial protein in another and healthier part of the body leads to the production, in this locality, of a specific ferment that is conveyed to the diseased area by way of the circulatory system, and aids in destroying the protein of the infecting micro-organism and its tissues.

The Non-specific Activity of Bacterial Vaccines.—Owing to the weight of laboratory investigations and the fundamental laws of specificity in immunity reactions, the sole efficacy of a vaccine has been generally ascribed to the production and activity of specific antibodies, and any deviation from this current of thought has been received with a measure of skepticism and disapproval. Scattered throughout the literature are the reports of more or less isolated observations that in prophylactic and therapeutic immunization good results have been observed in the prevention and treatment of diseases other than that specifically concerned.

The growing importance of this non-specific or "collateral" immunization by bacterial vaccines in the treatment of disease has been especially well summarized by Wright¹ as follows:

"I confess to having shared the conviction that immunization is always

¹ Lancet, March 29, 1919.

strictly specific. Twenty years ago, when it was alleged, before the Indian Plague Commission, that antiplague inoculation had cured eczema, gonorrhea, and other miscellaneous infections, I thought the matter undeserving of examination. I took the same view when it was reported in connection with antityphoid inoculation that it rendered the patients much less susceptible to malaria. Again, seven years ago, when applying pneumococcus inoculations as a preventive against pneumonia in the Transvaal mines, I nourished exactly the same prejudices. But here the statistical results which were obtained in the Premier Mine demonstrated that the pneumococcus inoculations had, in addition to bringing down the mortality from pneumonia by 85 per cent. reduced also the mortality from 'other diseases' by 50 per cent. From that on we had to take up into our categories the fact that inoculation produces in addition to 'direct' also 'collateral' immunization. This once recognized, presumptive evidence of collateral immunization began gradually to filter into our minds. Among, I suppose, many thousands of patients treated by vaccine therapy in private and in hospital, it happened every now and then that a patient was treated with a vaccine which did not correspond with his infection, and that that patient indubitably benefited. Again, it was not an uncommon experience for the subjects of a very chronic infection (such as pyorrhea) who were treated first by a stock vaccine, and afterward with an autovaccine, to assert that they derived more benefit from, and to ask to be put back upon treatment by, the stock vaccine.

"From such cases hints are conveyed to us that there may exist a useful sphere of application for collateral immunization; and that such sphere may, perhaps, be found in those cases where the infection is of very long standing, and where the patient has become very sensitive to, and has probably come very near the end of his tether in the matter of immunizing response to the particular species or strain of microbe with which he is infected. It will, with regard to such patients, be remembered that they constitute the third of those three classes of cases to which I referred at the outset of this lecture as very intractable to vaccine therapy.

"We are, however, here considering primarily the question of principle; and in connection with this what is of fundamental importance is: that we should discard the confident dogmatic belief that immunization must be strictly specific, and that we should in every case of failure endeavor to make our immunization more and more strictly specific. We should instead proceed upon the principle that the best vaccine to employ will always be the vaccine which gives on trial the best immunizing response against the microbe we propose to combat."

This subject is discussed in more detail in the chapter on the Use of Vaccines and Other Protein Substances in the Treatment of Disease. In prophylactic immunization specific agencies are of more importance, but in therapeutic immunization both specific and non-specific agencies are brought into operation.

Active Immunization in the Prophylaxis of Disease.—Theoretically it would appear possible to actively immunize against all those infectious diseases in which the microparasites or viruses were procurable and capable of being prepared in vaccines. Practically, however, this has not been accomplished. Vaccines for smallpox, rabies, typhoid and paratyphoid fevers, diphtheria, and a few other infectious diseases of human beings, and for anthrax, black-leg, and a few other diseases of the lower animals, are known to engender the production of immunity principles and afford protection varying in completeness and duration. In other diseases prophyl-

active immunization has either failed or the results are doubtful due either to a failure or tardiness of the body cells to produce immunity principles, a lack of vaccinogenic activity of our vaccines or our failure to prepare and administer the vaccines in a proper manner.

As was stated in the chapters on Immunity, in the presence of an infection the host endeavors to protect itself and overcome the invaders by various means, among which are phagocytosis and the production of more or less specific antibodies that may neutralize the poisons of the parasite (antitoxins), directly kill or destroy them (bactericidans), or so lower their vitality or resistance that they are more easily phagocyted (opsonins or bacteriotropins).

During an infection one or more of these protective forces, or all of them, are brought into action. After the infection has been overcome, the antibodies do not always disappear at once, but remain for some time in the body fluids and gradually diminish, so that if the host is invaded by the same parasite, the antibodies are at hand immediately to overcome it and protect the host absolutely, or at least so to modify the pathogenicity of the parasite or neutralize its products that the host will suffer but mildly while the parasite is being finally destroyed. The concentration and duration of the various defensive forces or antibodies vary in different individuals and in different infections, so that the degree and duration of an active acquired immunity are variable factors. Nevertheless—and this is the basis of active prophylactic immunization—an animal or a person may have specific antibodies for a certain parasite, produced by its own cells, without actually or necessarily suffering from the disease, due to the effects of inoculation with the germ or virus in a modified or attenuated form. The dose of vaccine may be so controlled that general symptoms the result of stimulation of the body cells are slight or not at all apparent, and, by gradually increasing the dose, more and more antibodies may be produced until a high degree of immunity is secured.

For prophylaxis, or the prevention of a disease, active immunization is accomplished by the production of antibodies so that they may be at hand to overcome an infection if it should occur. For example, the antibodies specific against the virus of smallpox may be produced by inoculation with cowpox virus, so that for years the system will be protected against smallpox. Even if vaccination has been delayed until smallpox has actually been contracted, inoculation with cowpox virus early in the period of incubation so stimulates the body cells that sufficient antibodies are produced to modify and lessen considerably the virulence of the smallpox virus.

This is especially true in rabies, when the vaccine is given in such doses and at such intervals that sufficient antibodies are produced to neutralize the effects of rabic virus and actually to destroy it during the period of incubation or during the interval that elapses between the time of infection and the appearance of the symptoms. In this way the great majority of infected persons escape the sufferings of rabies by enduring the relatively slight discomfort consequent to a series of subcutaneous injections.

Active Immunization in the Treatment of Disease.—This is bacterin or vaccine therapy, a method that owes its origin to the researches of Sir Almroth Wright and his colleagues. It was originally employed in the treatment of those infections that showed a tendency to chronicity in which true toxins played little or no part. Since recovery from an infection is in general dependent upon the mechanical removal of the infecting agent, aided by antibodies that facilitate phagocytosis or directly destroy the invading bacterium and neutralize its products, Wright believed that in chronic

infections autovaccination, or stimulation of the body cells to the production of antibodies, by reason of the fact that it is irregularly timed, is generally insufficient or altogether absent. For these reasons he believes that any stimulus that will arouse the body cells to throwing into the circulation substances from the invading bacterium or diseased tissues, may result in increased antibody formation, followed eventually by clinical improvement or cure. In certain cases this stimulation may be secured by judicious massage or manipulation of the diseased part, by passive hyperemia (Bier), or by similar procedures.

However, if the micro-organism is obtained and cultivated artificially, it is possible, in many instances, so to modify or attenuate the organisms (usually by heat) that they may be reinjected into the patient in sufficient numbers to furnish the stimulus necessary for arousing dormant or uninvolved body cells to produce the opsonins and other antibodies necessary for overcoming the infection. In other words, with each infection the host endeavors to protect itself by producing antibodies. When the protection is insufficient, the infection will spread; when the antibodies are in excess, the infection is overcome; when the forces are about equal, a stage of chronicity may result in which the host becomes accustomed, as it were, to the invaders, and, while the infection does not spread rapidly, it does not, on the other hand, recede. In cases of the latter type an extra dose of bacterial stimulant (a bacterin) may arouse dormant or inactive cells to furnish an extra quantity of antibodies and thus turn the tide.

In therapeutic inoculation, therefore, the fundamental principle is to stimulate in the interest of the infected tissues the unexercised immunizing capacities of the uninfected tissues. This is especially true in chronic infections, when the use of a bacterial vaccine may be likened to the application of the whip to a lazy horse that is capable of further effort and work. In acute infections, however, while the cells are at work they may be capable of greater effort, but vaccines should be given cautiously, as they may, to use the same simile, act as a whip to a willing and well-worked horse that is unable to respond or does respond, with resulting disastrous overexertion.

It should be remembered, in this connection, that usual forms of treatment should be given while bacertin therapy is being instituted. For instance, it is useless to administer a vaccine to a patient with a suppurative fistula or sinus if an infected silk suture is directly responsible for the suppuration. The suture should be removed, if possible, and after this is done a vaccine may be of considerable aid in overcoming the coincident infection.

In every form of bacterial infection a certain degree of success may be credited to vaccine therapy due not only to specific effects, but to non-specific effects as well. Failure to elicit beneficial therapeutic results is likewise common, and the whole subject of vaccine therapy is exceedingly difficult to evaluate in an impartial manner.

Especially successful results have been obtained in furunculosis and acute inflammatory sycosis; in localized streptococcus cellulitis and lymphangitis; in bronchitis, colicystitis, gonorrheal rheumatism and tuberculous pharyngeal conjunctivitis, dactylitis, orchitis, and arthritis.

Vaccine Therapy of Acute Infections.—The question of vaccine therapy in acute bacterial infections, as pneumonia, typhoid fever, puerperal sepsis, etc., has long been a subject of discussion with widely divergent opinions and practice. In acute *localized* infections, as streptococcus or staphylococcus cellulitis, and lymphangitis, acute cystitis, etc., vaccine therapy has proved successful as an adjunct to treatment and clinical experience indicates that in acute *generalized* infections, as pneumonia and typhoid fever, the

early and careful administration of vaccines in small doses may be beneficial and a distinct aid in increasing resistance and recovery.

As stated above, it would appear that vaccines are especially useful in chronic bacterial infections and that in acute infections vaccines may be contraindicated because (a) of the danger of adding to the toxic substances to be combated; (b) of the danger of a "negative phase" or period of lowered resistance, and (c) of the delay (seven to ten days) in antibody production. These are factors not lightly to be ignored, but, on the other hand, should not be allowed to carry too much weight, inasmuch as ordinary bacterial vaccines in moderate dosage are not markedly toxic, beneficial specific and non-specific effects may result in a few days and before antibodies are demonstrable in the blood.

As will be discussed shortly the fear of the "negative phase" has been largely responsible for hesitation in the use of vaccines in acute infections. I must state that I have shared this opinion, but experience is showing that this danger has been overemphasized. Actual decrease in antibodies by the injection of vaccines has not been conclusively proved. However, the injection of a vaccine may elicit focal and constitutional reactions due to toxic split protein products derived from the bacteria and the absorption of focus poisons; these effects in the writer's opinion constitute the so-called "negative phase." Since these substances add to the toxemia and effects of an acute infection they are to be avoided as far as possible; therefore if vaccines are given in the treatment of acute bacterial infections extra caution is required in dosage and intervals of injection.

The Requisites for Success and the Causes of Failure in Vaccine Therapy.

—The success of vaccine therapy may be said to depend upon the administration of appropriate doses (a) to increase the defensive powers of the body and mainly the leukocytes and bacteriotropic substances—an increase of the *phylactic power* as stated by Wright,¹ and (b) to provide means for the transport and access of these agencies to the foci of infection—the *kata-phylaxis* of Wright.

The increase of defensive powers—the *epiphylactic* response of Wright—will be incomplete and interfered with in infections producing continuous constitutional disturbances and fever from continuous or frequently recurring auto-inoculation with bacterial products, as in pulmonary tuberculosis; in these diseases vaccine therapy is not generally successful. According to Wright the auto-inoculations must be abolished in order to obtain a free field for the employment of properly graduated doses of vaccine or for properly controlled auto-inoculations. When possible the focus of infection should be incised and evacuated or, when this is not possible, local or general (rest in bed) immobilization are required.

Anything that prevents *kataphylaxis* or the carrying of defensive agents to the foci of infection will reduce the value of vaccine therapy as "when the arterial supply is interrupted or is closed down by collapse or the body petrified by cold, and the alkalinity of the lymph is blunted off by acid metabolites derived from the muscles" (Wright).

Likewise a condition of negative chemotaxis or the *ecphylaxis* of Wright interferes with vaccine therapy; especially in septic wounds in which the leukocytes and protective substances of the blood are repelled by toxins or absorbed by bacterial products and a favorable culture-medium for bacteria created. For these reasons vaccine therapy may be unsuccessful in long-standing infections and especially in unopened abscesses and sloughing wounds.

¹Lancet, March 29, 1919.

Under these conditions vaccine therapy should be accompanied by measures to counteract negative chemotaxis and promote kataphylaxis or the access of leukocytes and other defensive agencies to the foci of infection. Wright has summarized these remedial measures as follows: (a) The evacuation of pus or other fluids and replacement with fresh lymph which may be assisted by (b) cupping, the application of hypertonic saline solution or of irritant solutions, as Dakin's fluid, hot fomentations, Bier's hyperemia and massage, which excite hyperemia with increased exudation of cellular and serous elements and aid in the diffusion of defensive and curative agencies.

Contraindications to Active Immunization.—It should be emphasized that a properly prepared vaccine is a potent substance capable, when given in excessive dosage or when otherwise injudiciously administered, of doing much harm. This is the main reason why vaccines should be used very cautiously, if at all, in the treatment of severe generalized infections. In passive immunization the conditions are different, as the body cells are not taxed, but rather, through the neutralization of the toxic substances which they are combating, they are relieved, and an antibody-laden serum may, therefore, be freely administered in severe infections.

In active immunization for therapeutic purposes the conditions should be carefully weighed and the treatment conducted by one who is qualified to judge of the potencies of harm and good in a vaccine, and who has had sufficient experience to guide him in dosage and frequency of inoculation, the main objects being to tide over and aid nature during an acute infection, and to arcuse and stimulate her during a chronic infection.

In prophylactic immunization the physician should satisfy himself that the patient has no latent or active infection that may be rendered worse during the temporary depression that follows inoculation. It is true that this depression is fleeting and temporary, and that the possible harm incurred may be far outweighed by the ultimate good, but vaccines should be given with proper discernment and not carelessly and injudiciously. These remarks have no relation to cowpox vaccination, where the good so far overbalances the possible harm that in general all persons should be vaccinated, especially if an epidemic is impending.

(a) *Tuberculosis.*—There is at present some discussion relative to the harm that may be caused in tuberculosis by typhoid immunization. Probably all will agree that a patient with an active and acute tuberculous lesion should be refused inoculation, but when the lesion is quiescent or healed, or in the early latent stage, it is indeed difficult to understand how a prophylactic dose of typhoid vaccine will do more or as much harm as an attack of tonsillitis, rhinitis, or some similar acute infection.

(b) In *diabetes, carcinoma*, and other debilitating conditions vaccines should not be administered unless the indications or requirements are unusually urgent.

(c) *Advanced nephritis*, especially parenchymatous nephritis, may be regarded as contraindicating the administration of a vaccine.

THE ADMINISTRATION OF A BACTERIAL VACCINE

Method of Making the Inoculation.—As the administration of a vaccine is frequently followed by a temporary depression of the resisting powers of the individual and a feeling of lassitude, the injections are, as a rule, best given during the afternoon and evening, the night's rest aiding in overcoming the depression. Since the determination of proper dosage rests mainly on the observation of such clinical signs and symptoms as temperature, pulse,

and local reaction at the site of the lesion, the patient should be watched during the following twenty-four to forty-eight hours.

Vaccines are best administered with the aid of a 1 c.c. all-glass syringe, furnished with a sharp platinum iridium or steel needle. These may be sterilized in boiling water for a minute or longer. After sterilization, the parts should be carefully adjusted and the syringe loaded.

The injections should be given at a point where the tissues are loose, where muscular action is not much in evidence, and where pressure by clothing or weight is not made. The most suitable localities are in front of the shoulder, at a site about $1\frac{1}{2}$ inches below the center of the clavicle; high up in the buttock, or in the side of the abdomen, about 2 or 3 inches inside the anterosuperior spine of the ilium. In the majority of cases the injection may be given under the skin of the arm in the neighborhood of the insertion of the deltoid muscle. The skin at the point of injection should be touched with tincture of iodine, which is removed after the injection has been given by washing with alcohol.

Both convenience and experimental work to test the comparative efficacy of inoculation into different tissues point to the subcutaneous tissues as the most suitable *site for inoculation*. The best method of procedure is to pick up a fold of skin between the finger and thumb, and then to push the needle well down into the middle of the fold, and slowly inject the fluid.

Since it is known that the power of response of the tissues to the stimulus of a vaccine is somewhat limited, it would seem advisable to choose a new site for each successive inoculation.

Vaccines are sometimes injected intramuscularly, in which case the muscles of the buttocks afford a suitable site.

Intravenous injections of typhoid and other bacterial vaccines are sometimes made and especially for the purpose of eliciting non-specific reactions in the treatment of arthritis. The technic is described in Chapter XL.

The Effects of Inoculation.—The *local effects* produced at the site of subcutaneous or intramuscular inoculation vary considerably, being influenced by the nature of the individual, the variety and amount of the inoculum, and the sensitiveness of the patient's tissues to stimulation. In the majority of cases the local reaction is limited to a very slight reddening of the skin around the puncture for an area of about 1 inch. In some instances occasionally encountered where a large number of typhoid inoculations have been made, the reaction after the first dose is more severe than after subsequent doses, and is accompanied by considerable edema, hyperemia, and pain.

The *focal effects* about the lesion are exceedingly important in determining the reaction of the patient, and serve as a guide to the adjustment of dosage and intervals. Where, in a case of furuncle, and appropriate dose of staphylococcus vaccine is administered, within a few hours increased hyperemia is seen around the focus, and there is a slight increase in the swelling. When very small doses are given, these focal symptoms may practically be absent, but, as a rule, a slight reaction does no harm, but serves rather to show that the vaccine possesses some degree of potency and may aid in the curative process.

The *constitutional effects* may also vary within wide limits. An adequate, but not excessive, dose may, within a few hours, produce a feeling of lassitude, headache, slight rise in temperature, and acceleration of the pulse-rate. Severe constitutional reactions are generally due to excessive dosage, but may occur in some persons after doses that were previously well borne.

Frequency and Dosage of Inoculation.—No definite rules can be laid down, each patient being a law unto himself. The opsonic index has been largely abandoned as a guide to the administration of vaccine, the reaction and condition of the patient now governing the dosage. In more acute infections, and in delicate persons, smaller doses are usually indicated. It is well to make the first dose small, and if no reaction occurs within forty-eight hours, a second and a larger dose may be given. If, however, the patient presents other symptoms of a general reaction, the dose given was large enough, and may be repeated, as necessary, at intervals of from five to seven days. It should be carefully borne in mind that an increase in dosage is contraindicated so long as any sign of general or focal reaction is produced and steady progress is maintained. One should always be on guard to detect any signs of fresh infection by some other organism, and if a given vaccine is failing to exert a beneficial effect, additional cultures should be made, instead of continuing to administer dose after dose of the same vaccine.

The *intervals at which injections are to be made* are of some importance. It is certainly better to wait too long than to inoculate prematurely, but the ghost of the "negative phase" is always too prominent in the minds of the inexperienced. The inoculations may be given while improvement is still in progress or convalescence well established, in the endeavor to secure a summation of positive phases of clinical improvement; or one may wait for the first signs of retrogression before administering another dose. The former method is the preferable procedure, but is difficult to accomplish; the latter is less ideal, but is easier to perform and more devoid of risk.

The *dosage* varies according to whether the infection is acute or chronic, the nature of the micro-organism, and the age of the patient. No fixed rules can be given. In acute infections the dose should be small and may frequently be repeated; in chronic infections larger doses may be given at longer intervals. If in doubt as to the size of the dose to be given, it is better to give a small dose, and carefully observe the effect on the patient, letting this serve as an index to subsequent doses. Children tolerate relatively large doses of bacterial vaccines, but the dosage should depend on the weight and not on the age of the child.

The following is a list of the ordinary doses for adults of various bacterins:

Staphylococcus aureus.....	100,000,000 to 1,000,000,000
Staphylococcus albus and citreus.....	200,000,000 to 1,000,000,000
Streptococcus pyogenes.....	25,000,000 to 200,000,000
Gonococcus.....	25,000,000 to 200,000,000
Typhoid bacillus.....	250,000,000 to 1,000,000,000
Colon bacillus.....	100,000,000 to 1,000,000,000

The Mechanism of the Reaction Following the Subcutaneous and Intramuscular Injection of Vaccines.—The *local reactions* following the subcutaneous and intramuscular injection of vaccines may be due to a variety of factors as (a) irritation caused by the phenol or other preservative; (b) irritation caused by bacterial products; (c) irritation caused by the products of bacterial proteolysis, and (d) a local anaphylactic reaction to the bacterial protein.

The amount of phenol or other preservative added to a vaccine should be the minimum required, as 0.25 to 0.3 per cent., in order to avoid the irritation excited by larger amounts.

The irritation excited by the bacterial products varies considerably with different vaccines, being especially marked with vaccines of dysentery bacilli. These irritant substances are probably split bacterial proteins rather than

preformed bacterial substances and appear in old vaccines to a larger extent than in freshly prepared vaccines. Vaccines prepared by heating broth cultures and employing the broth as a diluent are usually more irritating than suspensions in saline solution. A part of the local reaction may be due to the digestion of the bacterial proteins by tissue and leukocytic proteases with the production of toxic split products exciting large and diffuse areas of pale erythema analogous to the non-specific reaction excited by the protein constituents of the diphtheria bacillus and broth in the Schick reaction.

In some instances the reaction is doubtless in part, at least, a local anaphylactic reaction to the bacterial proteins. This is particularly true in tuberculosis after the injection of tuberculin and in other chronic infections, as bronchitis and bacterial bronchial asthma, in which cutaneous and intracutaneous tests with bacterial proteins elicit positive reactions. It is not uncommon to observe that local reactions are of increased severity after several doses have been given, and these suggest the possibility of anaphylactic sensitization; likewise the second course of typhoid inoculations one or more years after a preceding course may elicit well-marked reactions of anaphylactic nature. Similarly the development of local reactions during the course of rabies vaccination suggests the possibility of these being due to active sensitization to the protein constituents of the spinal cord or brain substance of rabbits employed for the preparation of the vaccine. There can be no doubt that bacterial anaphylactic reactions may occur, and especially after the intravenous injection of vaccines, as discussed in Chapter XL. The subject of bacterial anaphylaxis in general is considered in Chapter XXIX.

The *focal reactions* occurring about the foci of infection are commonly in the nature of hyperemia with serous and cellular exudation, and are especially well marked in tuberculosis following the subcutaneous injection of an adequate amount of tuberculin.

These reactions are generally regarded as allergic or anaphylactic in character due to sensitization of the granulation and other tissues about inflammatory foci by bacterial proteins, the hyperemia being caused by an allergic reaction. For this reason these reactions have been considered strictly specific and diagnostic, and especially in tuberculosis, but within the last few years it has been shown that reactions of this kind may be elicited by non-specific agents and especially when these are injected intravenously (discussed in more detail in Chapter XXXIX). These focal reactions are very probably of therapeutic value by reason of the induced hyperemia and serous and cellular exudation resulting in the bringing in to the focus of leukocytes and bacteriotropic substances and the production of fibrous tissue, the latter being especially favorable for the walling off of tuberculous lesions.

The *constitutional reaction* is ascribed to the influence of protein constituents by Schittenhelm and Weichardt,¹ and particularly of the diamino-rich complexes, such as histones and protamins, which, according to Ruppel, are present in large amounts in a number of bacteria. Petersen² has grouped the toxic split products of bacterial proteins as follows: (a) Preformed protein split products which are toxic; (b) protein split products formed as the bacterial protein is fragmented in the host; (c) toxic growth products derived from the bacterial metabolism and excreted, and (d) toxic metabolic products derived from the pathologic cellular metabolism of the invaded organism.

¹ Münch. med. Wchn., 1910, lvii, 1769; 1911, lviii, 840; 1912, lix, 67; 1919, lxvi, 1403.

² Protein Therapy and Non-specific Resistance, MacMillan Co., 1922, 99.

In addition to the toxic split products of the bacterial vaccine it is likely that the absorption of toxic substances from the foci of disease mentioned above are important in this connection and capable of adding to the symptoms of fever, chill, tachycardia, malaise, and other symptoms. This absorption of toxic foci poisons is facilitated by the hyperemia and the poisons regarded by many as the cause of the constitutional reaction following the injection of tuberculin.

The Question of the Negative Phase.—In 1901 Wright¹ described what he termed the “negative phase” (since renamed by him “apophylactic phase”) following the injection of a large dose of typhoid vaccine—a temporary period in which there was diminished blood resistance or lowered immunity. This period of lowered resistance is believed to last from several hours to a day or more, followed by the positive phase of increased resistance. Wright also found that this lowered resistance was most pronounced after the injection of large doses or giving vaccines at too short intervals.

These views have greatly retarded the employment of vaccines in the treatment of acute bacterial infections. Wright and von Wassermann and Sommerfeld² maintain that injections of vaccines may decrease the antibody content of the blood of man and the lower animals; Bull,³ however, found that the administration of typhoid vaccine to rabbits did not cause, as far as could be determined, a decrease in the antibody content of the blood; indeed, after intravenous injections there was an increase of the normal antibodies which is particularly significant in connection with the subject of the effects of non-specific protein therapy.

However, as previously discussed in connection with vaccine therapy in acute infections, the reactions caused by vaccines due to the absorption of toxic split proteins from the bacteria, in addition to those from the foci of infection, may add to the degree of intoxication induced by a bacterial infection. These constitutional and focal effects may still further reduce resistance and constitute a real “negative phase,” not in the original meaning of Wright ascribed to the reduction in antibodies, but to increased protein intoxication. For this reason the dosage and intervals of injection of vaccines in the treatment of both acute and chronic infections, and for prophylaxis in the presence of epidemics, should be such as to avoid severe reactions.

¹ *Lancet*, September 14, 1901; *Brit. Med. Jour.*, 1903, 1, 1069.

² *Med. Klinik*, 1915, 11, 1307.

³ *Jour. Exper. Med.*, 1916, 23, 419.

CHAPTER XXXV

PROPHYLACTIC ACTIVE IMMUNIZATION OR VACCINATION

VACCINATION AGAINST SMALLPOX

Immunity in Smallpox.—Smallpox occurs spontaneously only in man, although some of the lower animals may be infected experimentally. Instances of natural immunity to this disease are rare; not infrequently, however, persons are encountered who are apparently resistant or immune to repeated cowpox vaccination, and in some instances these are known to have escaped smallpox infection. In the great majority of such instances of resistance to first vaccination failure is due to the use of a faulty virus rather than to actual natural immunity.

Unvaccinated human beings of all ages and both sexes are extremely susceptible to smallpox. The mortality among the aborigines and negroes is especially high. The child *in utero* may be infected by placental transmission of the virus providing the mother has smallpox.

One attack of smallpox usually protects for life, but second and even third attacks are known to have occurred. After recovery from smallpox immunity principles may be found in the blood. Attempts toward inducing passive immunization of animals against cowpox and smallpox by means of the injection of blood from vaccinated animals have been occasionally successful; likewise in a few experiments the viruses of cowpox and smallpox have been successfully neutralized or destroyed *in vitro* through contact with the blood-serum of vaccinated animals and human beings recovered from smallpox. The presence of complement-fixing antibodies in the sera of human cases of smallpox and of vaccinated calves has been reported by Jobling,¹ Sugai,² Dalm,³ Kryloff,⁴ Teisser and Gastinell,⁵ Klein,⁶ Konschegg,⁷ the writer,⁸ and others.

History of Vaccination.—Just when and where smallpox vaccination was first practised is not known. The original method of inducing immunization against the disease by introducing the virus from a smallpox patient into a healthy person through an abrasion of the skin and thus greatly diminishing the virulence of the disease was practised by the Turks during the eighteenth century, the chief object to preserve the beauty of the young Turkish and Circassian women. In 1878 Lady Mary Montagu, the wife of the British Ambassador at the Ottoman court in Constantinople, observing this practice among the Turks, had her own son and daughter inoculated and was largely instrumental in establishing the practice in Europe.

As regards the prophylactic value of this method of inoculation in England and continental Europe, statistics are incomplete, but the literature of contemporary writers shows that protection was usually complete. The induced disease was not, however, always mild, and not infrequently assumed an unexpected virulence that not only proved distressing and even fatal to inoculated individuals, but also constituted a source of infection to a community. While, therefore, the underlying principles were sound, and while

¹ Jour. Exper. Med., 1906, 8, 707.

² Centralbl. f. Bakteriöl., 1909, 49, 650.

³ Centralbl. f. Bakteriöl., 1909, 51, 136.

⁴ Centralbl. f. Bakteriöl., 1911, 60, 651.

⁵ Ibid., 1912-13, ref., 55, 555.

⁶ Münch. med. Wchn., 1914, 61, 2270.

⁷ Münch. med. Wchn., 1915, 62, 4.

⁸ Jour. Immunology, 1916, 1, 59.

these early attempts at preventive immunization mark an epoch in the history of medicine and of the world, it was not until Edward Jenner made his investigations into a theory held by farmers and by experimental evidence established it as true that a satisfactory method of immunizing the body against smallpox was introduced.

The peasantry in various parts of Europe, and especially in England, had generally observed that those who had had sores on their hands contracted from similar lesions on the teats of cows, usually escaped smallpox infection when the disease was epidemic in a community. In fact, it is said that several farmers deliberately inoculated members of their family with cowpox lesions and that these escaped smallpox.

Edward Jenner was a physician practising in Berkeley, Gloucestershire, and frequently used the method of direct inoculation from a mild case of smallpox among his patients. While a student he was impressed with the traditions of cowpox vaccination, and finding that they were largely true, determined to make experimental tests. On May 14, 1796 he vaccinated a boy, James Phipps, with virus from a cowpox lesion on the hand of a dairy maid, Sarah Nehnes, and on July 1st he inoculated the same boy with pus from a smallpox patient without resulting infection. In 1798 he furnished further proof that cowpox will afford protection against smallpox by inoculating a child direct from a vesicle on the teat of a cow, and continued the inoculation from arm to arm through a series of 5 children, after which all were inoculated with smallpox virus, without a single case developing. In the same year he published "*An Inquiry into the Causes and Effects of the Variolæ Vaccinæ*," illustrated by four plates, and within a year or two vaccination became general over Europe.

Vaccination was introduced into the United States in July, 1800, by Dr. Benjamin Waterhouse, Professor of Physics at Harvard University, who vaccinated his own children. At about the same time John Redman Coxe, of Philadelphia, vaccinated his oldest child and then tested the experiment by exposing him to cases of smallpox. This bold repetition of Jenner's experiment considerably strengthened public confidence in the method and the practice spread rapidly. Thomas Jefferson, writing in 1806 to Edward Jenner, said: "Future generations will know by history only that the loathsome smallpox existed and by you has been extirpated."

But Jenner and his earlier supporters met with much opposition, often bitter and unrelenting, and this is readily understood when it is realized that even at the present day, over a hundred years later, cowpox vaccination still has its opponents, in spite of the fact that the value of the method has been established, and it has been found the greatest of all boons to the human race, and notwithstanding that it has been definitely proved that a thorough and continuous practice of the operation would quickly eradicate smallpox from the face of the earth. This opposition is especially pernicious and unjust, since the practice of former years of vaccinating by direct transmission from arm to arm has been entirely abandoned, and that animal lymph, prepared and collected under strict aseptic precautions, is being used exclusively.

The Relationship of Variola and Vaccinia.—The relationship of variola to vaccinia has been discussed since Jenner's time, but no adequate explanation has been found.

According to the general belief the smallpox virus, whatever it may be, is altered in its passage through a lower animal, and loses forever its power of producing smallpox, but is still so closely related that the antibodies it produces are sufficient to protect against smallpox.

The close interrelationship existing between vaccinia and variola is shown by several facts. *First*, by the presence in the virus of both, and in section of the skin of both, of microscopic cell inclusions, first described by Guarinieri in 1892. This finding has been confirmed by Pfeiffer in Germany and Councilman and his associates in this country. These investigators have made extensive studies of these bodies, and believe them to be protozoa intimately associated with the etiology of vaccinia and variola. More recently Fornet has described certain small, diplococcus-like bodies that were found in cowpox vaccine and in smallpox lesions. These are regarded as having an etiologic relationship to smallpox, and if these findings are confirmed, would prove the identity of variola and vaccinia.

Second, recent investigators, particularly Copeman, of England, and Brinkerhoff and Tyzzer, of America, have shown, by carefully conducted experiments, that vaccination will protect monkeys against subsequent inoculation with smallpox virus, and this completely confirms the early experiments of Jenner and others who proved the efficacy of vaccination by the "variola test."

Third, smallpox inoculated into calves is said by some investigators to produce a disease similar to cowpox after passage through several animals.

The Preparation of Cowpox Vaccine.—During the early days of vaccination it was customary to inoculate human beings with material obtained from the pustules of those previously vaccinated. The old-time physician carefully removed choice scabs and carried them about in a special case ready for inoculation. While this method served its purpose well, there were several drawbacks to its use, the chief of which was the danger of transmitting syphilis. It has now for many years been the custom to use virus obtained from animals, the production of which can be carefully controlled and tested, any danger of transmitting syphilis being thus obviated, because the heifer or cow used in the preparation of the virus is not subject to this disease. The opponents to vaccination, however, persist in using old and obsolete statistics regarding the transmission of syphilis to support their claims, although these have absolutely no bearing upon the modern methods of preparing the virus.

Seed Virus.—This refers to the virus for vaccinating the calves or other animals used, and is a most troublesome factor to those engaged in this work. Park has found the following method the most economic, efficient, and reliable method found in the New York laboratories: "Crusts are collected from healthy children about nineteen days after successful vaccination. These crusts are cut up and emulsified with boiled water to a mucilaginous paste. This humanized seed is inoculated into an area about 6 inches square upon the abdomen of a calf, the remainder of the calf being vaccinated in the ordinary way. The pulp from this special area is separately collected and glycerinized in the usual way. It is then tested bacteriologically and clinically. This bovine virus from human seed is now used in a dilution of 1 part to 12½ parts of normal salt solution to vaccinate rabbits. The seed is rubbed thoroughly on the freshly shaved skin of the back. Five days after vaccination the pulp is removed with a curet, weighed, and emulsified in a mortar with the following solution: glycerin 50 per cent., sterile water 49.5 per cent., and carbolic acid 0.5 per cent., in the proportion of 1 part of pulp to 8 parts of the solution. Four rabbits should yield from 15 to 20 c.c. of this emulsion, an amount sufficient to vaccinate one calf."

Large amounts of vaccine are then prepared by vaccinating calves with this seed virus.

The New York Vaccine Laboratory produces a virus that is never more than four successive transfers from a human case of vaccinia, and is guaranteed to give 100 per cent. of "takes" in primary vaccination.

Animals.—Various animals have been used, but female calves from two to four months of age are preferable. Older animals may be used, and in several European institutes cows are usually employed. With properly constructed operating-tables they may be handled with comparative ease. Rabbits have also been used, especially in propagating the seed virus and to obtain pure and highly active viruses.

The calves are kept under supervision for at least a few days. In some institutes they are tested with tuberculin, although with good veterinary inspection this test is not necessary. Soon after admission the animal is clipped and given a thorough cleansing, which includes the feet and the tail.

On the day before vaccination is to be performed the belly wall is cleanly shaved from the cuneiform cartilage to the pubis, and well up on the inner sides of the thighs and the flanks. The skin is then thoroughly washed. Just preceding the vaccination the animal is fastened to the operating-table and the abdomen and inner surface of the thighs prepared as for an aseptic abdominal section, *i. e.*, a thorough scrubbing with hot water, green soap, and soft brush, followed by alcohol and sterilized water, the parts being then dried with a sterile towel. All other parts are covered with sterile sheets, and the calf is now vaccinated under aseptic precautions.

Vaccination.—About 100 small scarifications are now made in these areas, preferably by cross-scratches or in rows of lines about 1 to 2 cm. square and at least 1 to 2 cm. apart. The scarification is simple, but usually brings a small amount of blood. After they have been made, they are mopped with sterile gauze and rubbed with the charged slips, using one or two slips for each small area, depending on the amount of virus each slip contains. The lesions are allowed to dry, and are then covered with sterile gauze or a simple protective paste, or are left entirely uncovered.

Precautions should be taken to keep the animals as clean as possible. Inoculated animals are to be kept in stalls or stables apart from those under observation. The stable should be so constructed that the floors can be flushed daily with a hose and hot water. Excreta should be removed promptly. No bedding is permissible, and means should be provided for fastening the legs and preventing the animal from kicking the scarifications.

Collection.—Ordinarily, within forty-eight hours of vaccination, the scratches are pinkish, slightly raised, and papular, and within five or six days, depending upon the rate of development of the vaccine vesicles, the virus should be ready for collection (Fig. 169). The calf is killed and placed upon the operating-table. The appointments of the operating-room are usually equal to those in a well-equipped hospital operating-room, being supplied with all conveniences and means for carrying out a careful, painstaking, and aseptic technic.

The exposed parts are covered with sterile sheets. The operator and his assistant are clad in aseptic gowns. The vaccinated field is thoroughly scrubbed with soap, sterile water, and gauze, and mopped with sterile gauze. Crusts are carefully picked off, and the soft, pulpy mass cureted off with a special, spoon-like curet and collected in a sterile vessel. After the curetage, serum exudes from the excoriated base of the vesicle, and ivory tips may be charged in this. The sticky and pulpy exudate is then mixed with four times its weight of glycerin and water (50 per cent. glycerin, 49 per cent. water, 1 per cent. phenol), and this is done most effectively by passing the mixture between the rollers of a Doring mill. The glycerinated pulp is allowed to

stand for three or four weeks in order to allow bacteria, which are invariably present, to undergo dissolution. At the end of this time the glycerinated pulp is thoroughly titrated in specially constructed triturating machines, passed through 40- and 100-mesh sieves, and put up in small capillary tubes, which are sealed, or "vaccine points" may be prepared. If properly preserved in sealed tubes in a dark, cool place (-2 to 4° F.) the virus should remain active for at least three months.

According to Park and Huddleson, 10 grams of pulp and 200 charged slips would be an average yield from a calf, and when made up should



FIG. 169.—PRODUCTION OF COWPOX VACCINE.

Note the lines of cowpox lesions over the abdomen and flanks of the calf. The surgeon is about to cleanse this area in a thorough and careful manner, after which the cowpox material is removed with a curet and collected in a sterile vessel. All precautions are taken to insure as thorough aseptic technic as possible.

suffice to vaccinate at least 1500 persons. Calves vary greatly in their yield of virus. Of 2 calves vaccinated in exactly the same manner, one may furnish material for 500 vaccinations and the other for 10,000 inoculations.

Testing the Vaccine.—The virus may be tested for its efficacy by a variety of methods. Calmette and Guérin¹ inoculate rabbits upon the inner surfaces of the ears and estimate the potency of the virus from the speed of development and the size of the resulting lesions. Guérin² estimates the potency

¹ Ann. de l'Inst. Pasteur, 1901, 15, 161.

² Ann. de l'Inst. Pasteur, 1905, 19, 317.

of virus quantitatively by inoculating rabbits with serial dilutions ranging from 1 : 10 to 1 : 100. Fully potent virus should cause closely approximated vesicles in a dilution of 1 : 500, and numerous isolated vesicles in a dilution as high as 1 : 1000.

Quantitative estimation of the bacteria in the glycerinated virus is made by the plating method, and the vaccine used only when the numbers of bacteria have been greatly diminished or are entirely absent. The vaccine is also tested for tetanus by anaërobic cultures and by injecting relatively large quantities subcutaneously into guinea-pigs and mice.

Tests are also made for streptococci and *Bacillus welchii* (*B. capsulatus aërogenes*).

After all of these laboratory tests for purity have been made and found satisfactory, *and not until then*, the vaccine is submitted to clinical trial. Fifteen inoculations are made upon previously unvaccinated children. These must all show a perfect take in order to pass the vaccine as up to standard. A clinical test is made every two weeks thereafter as long as the vaccine is on the market. If one of these tests fails before the end of the period of guarantee, the vaccine is called in (Park).

Under the Federal Law of July 1, 1902, and the regulations framed thereunder, all firms manufacturing vaccine virus are required by the Secretary of the Treasury to obtain a license before they may sell their products in interstate commerce. The vaccine laboratories are carefully inspected by an official of the Hygienic Laboratory of the United States Public Health and Marine Hospital Service; the inspector carries away with him as many samples of virus as he wishes, and additional samples are purchased in the open market in different parts of the country. All these are subjected to a vigorous bacteriologic examination, especially for tetanus bacilli, by a laboratory worker who devotes all his time to this work. The federal regulations require each vaccine institute to perform a careful autopsy on each calf after the vaccine virus has been removed, and if any communicable disease is found or suspected in the animal, the virus must not be placed on the market, but must be destroyed. In accordance with this law, permanent records of the bacteriologic examinations of the virus and of the autopsy shall be kept in each institute.

Noguchi's Method of Preparing Cowpox Virus.—Noguchi¹ has succeeded in freeing vaccine virus from all associated bacteria by means of suitable disinfecting agents, and propagated the pure virus in the testicles of rabbits and bulls. The virus cultivated in this manner is not only devoid of bacteria, but appears capable of definite transfer from one animal to another. The multiplication of the virus within the testicle was found by Noguchi to reach a maximum on the fourth or fifth day after inoculation, with diminution after the eighth day. Skin lesions produced in rabbits and calves with the original and purified or bacteria-free viruses were found identical, and persons have reacted to the latter in an entirely typical manner. These results are of great value and importance and tend to increase the safety of vaccination and incidently weaken the arguments and contentions of antivaccinationists.

Unfortunately, however, the method has not proved successful on a commercial scale and the virus has been found by Force² and others to rapidly deteriorate.

Keeping of Vaccine.—Vaccine virus should always be kept at a low temperature (33° to 40° F.). *Not infrequently failure to successfully vaccinate an individual is due to deterioration of the virus kept in a warm place.*

¹ Jour. Exper. Med., 1915, 21, 539.

² Jour. Lab. and Clin. Med., 1917, 3, 220.

Technic of Vaccination.—The essential part of the process of vaccination is that the virus should be introduced through the epidermis so as to be absorbed by the lymphatics and blood-vessels of the corium.

The *site* usually chosen is the skin of the outer side of the upper arm, over the insertion of the tendon of the deltoid muscle. Sometimes, in females, the outer side of the thigh or well above the knee on the inner aspect of the thigh is used. Vaccination on the foot and leg, however, is never advisable, as it would appear that such vaccinations are more prone to take on an excessive inflammatory action owing to the greater congestion due to the dependent position of the lower extremities; there is also more likelihood of secondary infection and mechanical violence occurring.

Preparation of the Skin.—The skin should be washed with soap and water and in any case with alcohol on gauze or cotton, care being taken



FIG. 170.—TECHNIC OF VACCINATION.
First Step: Proper cleansing of the skin with alcohol and cotton.

not to rub too vigorously; if the skin is reddened, it is best to wait until the hyperemia subsides and always until the skin is dry (Fig. 170).

Several methods may be employed for vaccination as follows:

(a) *The Scratch Method.*—A drop of the virus is placed on the skin. If supplied in a capillary tube, it should be pushed through the small rubber bulb which accompanies it, wiped with alcohol, and the end broken off; the other end is broken off in the bulb and the contents expelled (Fig. 171).

The under surface of the arm is grasped with the operator's left hand so as to stretch the skin where the virus has been placed; with a sterile needle or other suitable instrument a scratch not deep enough to draw blood is made through the drop of virus, parallel with the humerus and about $\frac{1}{4}$ inch long (Fig. 172).

The virus is then gently rubbed in with the side of the needle or other smooth, sterile instrument, as a toothpick (Fig. 173). Some blood-tinged



FIG. 171.—TECHNIC OF VACCINATION.
Second Step: Applying a drop of virus to the skin.



FIG. 172.—TECHNIC OF VACCINATION.
Third Step: Abrading the skin with a needle through the virus.

serum may ooze through the virus, but this should not be sufficient to wash the virus out of the wound.

The virus should be allowed to dry on the abrasion for at least fifteen minutes.



FIG. 173.—TECHNIC OF VACCINATION.

Fourth Step: Rubbing in the virus followed by exposure for fifteen minutes for drying.

The vaccinal wound should now be covered with a square of sterile gauze fastened to the skin by adhesive strips (Fig. 174); if other shields are em-



FIG. 174.—TECHNIC OF VACCINATION.

Fourth Step: Dressing the wound.

ployed, care must be exercised in order that the wound shall be properly protected against contamination without maceration of the skin.

Cross-scarification, which is forbidden in Germany, favors the growth of anaërobic bacteria under the crust that forms on the surface of the abrasion where the resistance is lowered by the action of the virus. The circular scarification gives more control over the dosage, and there is no tendency to the development of excessively sore areas.

Usually one *inoculation* of the virus is sufficient, but in times of threatened epidemic two or more inoculations are made at the same time, not only to insure a successful result, but rapidly to immunize the patient. It would appear that the degree of immunity bears some relation to the number or size of the vaccination lesions, and this can readily be understood if the infection is local and the body cells are stimulated by a diffusible toxin. If, however, the vaccination lesion is but the point of entry of what becomes a general infection, then a small lesion should suffice. This point has not been definitely settled, but statistics tend to show that persons vaccinated in two or more areas develop an immunity more quickly and that this immunity is more lasting.

The subsequent care of the wound is of considerable importance. The operation is usually regarded as a trivial one, and justly so, but the lesion requires judicious after-treatment instead of being entirely neglected, as it so often is. The severe infections are usually attributable to gross and careless contamination of the wound. The best possible protection to the vaccinal ulceration is afforded by the formation of a hard, solid crust, due to desiccation of the contents of the vaccine vesicle and pustule. Unless undue inflammation and suppuration set in, such a crust will form. Care must be taken that the crust is not subjected to mechanical violence calculated to loosen or to detach it.

Constricting shields are likely to be unsatisfactory. The adhesion of the crust to the sleeve or to a piece of protective gauze will often lead to forcible decrustation when the sleeve or the gauze is removed. Schamberg and myself¹ have found that daily applications of a 4 per cent. alcoholic solution of picric acid upon the vaccinated area after the first forty-eight hours does not interfere with the success of the vaccination, and lessens the degree of local inflammatory reaction and constitutional disturbances by hardening the epithelial covering of the vaccine lesion, and thereby decreasing the liability of extraneous bacterial infection.

A point to be emphasized is that severe lesions are unnecessary, and are usually due to scratching of the vesicle or pustule and consequent introduction of dirt. No doubt tetanus bacilli may be introduced in this manner, the resulting scab affording the necessary anaërobic conditions for their development.

(b) *The Drill Method.*—The arm is cleansed as described. A von Pirquet drill shaped like a very small screw driver with a moderately sharp end not more than 2 mm. wide, is sterilized by dipping in alcohol and flaming. The skin is drawn taut, the drill held between the thumb and middle finger and with a twisting motion and moderately firm pressure, one or two small circular abrasions are made without drawing blood (Fig. 175). A drop of virus is then added and gently rubbed into the skin by means of the drill or wooden applicator; or the virus may be first placed on the skin and the abrasions made.

This method is especially recommended by Force,² who makes three abrasions and applies virus to each.

(c) *The Multiple Acupuncture Method.*—This method has been especially

¹ The Lancet, London, November 18, 1911, 1397.

² Jour. Lab. and Clin. Med., 1916, 2, 597.

recommended by Hill.¹ The skin is cleansed and three small drops of virus are placed at least 1 inch apart.

The vaccinator's hand is closed on the arm from behind, so as to draw the skin tight in front, and a sewing needle point, held slanting nearly parallel with the arm, is pressed against the skin through the drop of vaccine. Then it is that 1/1000 inch of the point sticks through the upper layer of the skin, carrying the vaccine with it. The needle is instantly withdrawn and similar punctures are made beside each other until a dozen are made in the space of 1/16 square inch or less. The two other areas are pricked in the same manner. The excess of vaccine is wiped away and the arm dressed as described.

(d) *Gauze Method*.—With nervous patients the abrasion may be made by gauze drawn over the index-finger and held by the thumb and other fingers; the selected point is briskly rubbed until slightly abraded over an area about



FIG. 175.—TECHNIC OF VACCINATION EMPLOYING THE VON PIRQUET SKIN BORER FOR SCARIFYING THE SKIN.

the size of a thumb-nail. The virus is then deposited and allowed to dry. The wound is then dressed in the usual manner.

(e) *Intracutaneous Method*.—Wright² has described a method consisting of diluting the virus as ordinarily supplied with an equal amount of sterile water and injecting 0.1 c.c. intracutaneously by means of a tuberculin syringe and a small needle (gage No. 26). The technic of injection is exactly similar to the Schick test.

(f) *Subcutaneous Method*.—Goodale³ has recommended the subcutaneous injection of about one-half to three-quarters of the usual amount of virus supplied in a capillary tube, diluted with sufficient sterile water to make

¹ Brit. Med. Jour., 1917, 1, 189.

² Jour. Amer. Med. Assoc., 1918, 71, 654.

³ Amer. Jour. Med. Sci., 1919, 158, 721.

1 c.c. Since there is no open wound, dressings are not required and the dangers of secondary infection are practically eliminated, the operation, of course, being conducted with the usual aseptic precautions. The main objection to the method is that a scar does not follow and the presence of a typical scar affords the only indisputable evidence of successful vaccination in subsequent years.

Internal administration of vaccine by swallowing the virus generally fails and should never be practised. Garrison¹ reported that of 25 persons given vaccinum by mouth, 20 were subsequently proved to be unprotected, as they were vaccinated successfully by the ordinary or scarification method. Force, likewise, has never observed the slightest immunity following the internal administration of virus.

Precautions.—While vaccination is a slight operation, it demands skill in performance and care in after-treatment in order to avoid the rare, but serious, complications. For the prevention of these complications vaccination (*a*) should be conducted with strictly aseptic technic, (*b*) cross scarifications should not be made, (*c*) the inoculation should cover a small rather than large area, although two or three areas may be inoculated at one time and (*d*) the wound should receive a proper primary dressing, and subsequent care to avoid secondary infection. On account of possible fouling by perspiration and to lessen the chance of contamination by street dirt, vaccination should be performed preferably in winter as emphasized by Elgin.² The prevention of secondary infection depends essentially, according to Force and Stevens,³ on the use of small multiple scarifications which give rise to *small vesicles* which are *not easily broken*, and do not foster anaërobic conditions and a central slough.

Age for Vaccination.—Ordinarily children should be vaccinated when about six months of age, again at six years, and preferably at puberty. At times of threatened epidemics everyone should be vaccinated and even children under four months of age. There is no special reason why healthy infants under four weeks of age should not be vaccinated.

Failure of Vaccination.—When primary vaccination fails it is generally due to a weak or dead vaccine or defective technic. True natural immunity is rare, although repeated vaccinations with inert virus may result in the production of some immunity. *A common error is to use vaccine that has been improperly preserved*, usually one that has been allowed to remain at ordinary room temperatures for sufficient time to weaken or destroy the virus. Deep scarification with bleeding may prevent the absorption of sufficient virus. I have known adults to prevent successful vaccination by immediately washing the skin and applying a bichlorid dressing. With scarification methods, at least, a few minutes should be allowed for absorption before the excess of vaccine is removed.

Subsequent Examinations and Reactions.—With individuals vaccinated for the first time and who have never had smallpox, the vaccination should be inspected about one week later, at which time the "take" will be well developed or clearly a failure.

With individuals who have been vaccinated before, the site should be inspected twenty-four hours later and again on the fifth day. If there is redness with or without a papule which has subsided on the fifth day, it is considered a *reaction of immunity*. This reaction may be caused by inert or dead as well as by living vaccine, and indicates the presence of antibodies

¹ Jour. Amer. Med. Assoc., 1917, 68, 979.

² Amer. Jour. Public Health, September, 1915.

³ Jour. Amer. Med. Assoc., 1917, 68, 1247.

in the tissues; if a vaccine has been employed which is known to be active on the basis of successful "takes" among others, revaccination is unnecessary.

If redness on the first day is followed by the development of a small vesicle by the fifth day, *which rapidly subsides*, the reaction is designated as *vaccinoid*, which is mild or secondary vaccinia.

If there is practically no change until the third day, when an areola develops, *vaccinia* will develop.

The Mechanism of Smallpox Vaccination.—In smallpox the infection is general and the virus is distributed to the skin, mucous membranes, and internal organs by the blood; this is indicated by the successful inoculation of animals with the blood, bone-marrow, and other tissues of the smallpox victim.

In vaccination the living virus of cowpox is deposited in the epidermis where it survives, multiplies, and produces the local lesion and some general effects designated as vaccinia. After vaccinia the individual is found immune to smallpox because the virus of cowpox or vaccinia has induced the production of antibodies which destroy and protect against the virus of smallpox.

It is debatable whether the virus of cowpox finds its way into the blood and internal organs during vaccination (vaccinia). Some investigators claim that this occurs; others claim that it does not on the basis of failure to vaccinate animals with the blood and extracts of the internal organs from animals with vaccinia.

The antibody may occur in the blood because the blood or serum of the individual recovering from smallpox, as likewise the blood or serum of the human being or lower animal after vaccinia when mixed with the virus in a test-tube, is known to be able to kill the virus. Furthermore, Jobling¹ and others, including the writer,² have found specific complement-fixing and other antibodies in the sera after smallpox and vaccinia.

It is highly probable that antibodies are engendered by epithelial cells as well as by the bone-marrow and the tissues of other organs. Doubtless the virus produced in the vaccinal lesion enters the body fluids either, in a living state or dead. If living the amount present in the blood at any time must be small because vaccinal lesions rarely occur in other parts of the body, even in eczema, if direct inoculation is prevented.

Apparently the epithelium is principally immunized. These cells are able to destroy the virus of smallpox or cowpox upon direct inoculation because antibodies (both destructive and allergic) occur in them. Smallpox virus is probably inhaled and enters by way of the lymphatic and vascular channels of the upper respiratory tract, unless the epithelial cells of the respiratory mucosa are immunized by a previous attack of smallpox or by vaccinia. Possibly the antibodies sometimes present in the blood are capable of destroying the virus upon intravenous injection, but it is more likely that the viruses of smallpox and vaccinia are strictly dermatropic and fail to infect if the epithelial cells are immune.

The Phenomena of Vaccination; Vaccinia.—Immediately following vaccination a slight redness appears, which usually subsides rapidly. After a short period of incubation—on or about the *third day*—a *slight red elevation* makes its appearance, and the lesion begins to burn and itch. On the *sixth or seventh day* the abrasion becomes a *small, silvery gray, umbilicated vesicle with a sharply raised edge*, filled with a clear serum, and surrounded by a narrow red areola (Fig. 176). By the *tenth day* the characteristic features are more marked, and the lesion has usually reached its height, being

¹ Jour. Exper. Med., 1906, 8, 717.

² Jour. Immunology, 1916, 1, 59.



FIG. 176.—VACCINIA (SEVEN-DAY LESION).



FIG. 177.—VACCINIA (NINE-DAY LESION).

FIG. 178.—VACCINOID. A VACCINATION SCAR.

Fig. 176 shows a seven-day vaccination vesicle. Fig. 177 shows a nine-day vaccination vesicle just before pustulation occurred. Fig. 178 shows a recent vaccination scar with pitting and radiation, also a three-day "vaccinoid" or "immunity reaction" with a small vesicle.

accompanied by a burning sensation and an almost uncontrollable desire to scratch (Fig. 177). The areola is now quite angry in appearance, and numerous minute vesicles are seen on its surface. By the twelfth day the areola is smaller, the contents become turbid and commence to dry, so that a few days later a scab has formed that drops off in another week or two.

About the fifth day the child becomes restless and irritable and shows a slight elevation of temperature. These symptoms may become more pronounced until the end of the second week, when they subside rapidly.

Precautions should be taken to prevent scratching and infection of the vesicle. The old-time "beautiful arms," with well-marked cellulitis and adenitis of neighboring glands, were largely due to secondary infection, and are not at all necessary in the process of vaccination. Evidence would tend to indicate that the vesicle is the typical lesion of both smallpox and vaccinia, and that the pustules are simply infected vesicles. Ordinary surgical care will do much to rob vaccination of its discomfort and to render the operation a most harmless one.

Immunity Reaction; Vaccinoid.—The results of a vaccination, therefore, can be inspected and verified on or about the seventh to the ninth day. With persons who have been vaccinated successfully on a previous occasion the vaccinated area may show a slight areola at the end of twenty-four hours, with or without a papule, which subsides in seventy-two hours. This is called a "reaction of immunity," and is due to the presence of antibodies against the virus. Or a small, itchy, burning papule may form, which develops into a small vesicle maturing on the fifth or sixth day, and then rapidly subsiding, constituting the reaction known as *vaccinoid* (Fig. 178). Occasionally vaccination is followed by the appearance of various eruptions.

Vaccination Scar.—The appearance of the *scar* varies according to its age and to the degree of tissue destruction. The physician is not infrequently requested to examine a person and determine if the scar is satisfactory evidence of successful vaccination. The typical *good scar* is circular, and about the size of a ten-cent piece, with smooth, white, and depressed center and a raised border. The border shows numerous radiations, and the entire scar may show little pits of former hair-follicles when the lesion was sufficiently destructive to remove the upper portion of the corium (Fig. 178). A burn or an ordinary pyogenic infection may leave scars quite similar to those of vaccination, and vaccination scars may show wide variation, but the circumscribed character, the raised border with radiations and depressions, and the appearance of having been stamped on the skin by a sharply cut die are quite characteristic. *Poor scars* are those that were said to have been the result of vaccination, but in very many instances they are so indistinct as to make it difficult or impossible to recognize them as vaccination marks.

Vaccination Certificates.—Physicians should exercise great caution in the issuing of certificates certifying to immunity. Mere failure to secure typical vaccinia by vaccination or repeated vaccination is insufficient. If there is typical vaccinia or a satisfactory scar of previous vaccination with the development of an "immunity reaction" or vaccinoid on revaccination, a certificate may be issued. Otherwise revaccination should be repeated until one of these three reactions are observed before a certificate may be properly issued. Force, who has given this subject particular study, has stated that he has never yet seen the naturally immune individual, and that there will be no failures if cold, fresh, potent vaccine is used.

Revaccination.—One successful vaccination does not necessarily confer an absolute immunity against smallpox, and failure to recognize certain limita-

tions in this respect has done harm by enabling antivaccinationists to create a distrust in the minds of the ignorant by pointing to individual instances of failure. That a person who has once been vaccinated may afterward suffer from smallpox is undoubted, but usually the vaccination was performed many years previously, and in any case the disease, when it does occur, is relatively mild (varioid).

There can be no doubt that the immunity gradually diminishes. Force¹ found that approximately 14 per cent. of persons successfully vaccinated ten to twenty years previously and showing scars, developed vaccinia and 38 per cent. vaccinoid, upon revaccination. After twenty years approximately 35 per cent. developed vaccinia and 27 per cent. vaccinoid. Vaccinia among these individuals upon revaccination indicated considerable loss of immunity to cowpox virus, although such individuals exposed to smallpox frequently escape, whereas persons never successfully vaccinated almost invariably contract the disease. Vaccinoid indicated still less loss of immunity.

Perhaps seven years may be taken as the average period of fairly complete protection. Children should be vaccinated within the first year after birth, revaccinated upon entering school, and again after leaving it. If smallpox is prevalent, all persons should be vaccinated, regardless of the fact that they have previously been vaccinated. Only those who have had smallpox may be excused. If, as a matter of fact, persons are still immune, the vaccination will not "take" and no harm is done, whereas, if it succeeds, such persons will have the satisfaction of knowing that their immunity has been increased. Hence it cannot be too strongly emphasized that not only vaccination, but revaccination, is indicated to protect the individual and society against smallpox. Dwyer claims that a person should be revaccinated repeatedly in succession until he fails to react; even a slight "take" would indicate incomplete immunity.

Occasionally a non-immunized person refuses to "take," but vaccination should be repeated three or four times, as failure is not infrequently due to old and inactive virus, and the actual number of persons absolutely insusceptible is very small indeed.

Risks of Vaccination.—When vaccination is properly performed with a good virus, the risk of permanent injury to life or limb is almost negligible.

1. *Tetanus.*—The most serious of the injuries that have been attributed to vaccination is tetanus. The tetanus bacillus and its spores are so widespread in nature that opportunity presents itself for contamination of the vaccinal wound and the virus itself. Every precaution should, therefore, be taken in the preparation of virus, and it is especially important that physicians and laymen should realize the necessity for observing ordinary care, and at least ordinary cleanliness, in the treatment of the vaccinal wound.

It is exceedingly difficult to determine the source of infection in each case of vaccinal tetanus, but experimental investigations would tend to indicate that the virus itself is seldom, if ever, the vehicle of infection. Anderson² has stated that in experiments carried out by Francis³ on monkeys and guinea-pigs with vaccine lymph purposely contaminated in the laboratory with countless numbers of tetanus spores, it was found impossible to communicate tetanus in this manner, although the vaccinations were more severe than the ordinary vaccinations performed on man, in that several

¹ Jour. Lab. and Clin. Med., 1917, 3, 220.

² Public Health Report, 1915, 30, No. 29.

³ Bull. Hyg. Lab., 95, August, 1914.

places were inoculated and the areas abraded were large. Anderson, furthermore stated that his "conclusion from these experiments is that it is almost impossible to produce tetanus, even with vaccine virus that contains tetanus germs in it, by the simple act of vaccination."

Since 1909 there were approximately 100,000 specimens of vaccine virus examined in the Hygienic Laboratory, particularly with the purpose of determining the presence of tetanus germs or their products. The vaccine was purchased in the open market, and the examinations were made as thoroughly as it was possible to make them. To use Anderson's words: "We have never succeeded in finding any evidence of the presence of the tetanus organism or its products in vaccine virus."

It would appear, therefore, that virus prepared according to modern methods and with all recognized precautions is safe. In view of the incidence of tetanus following other injuries, it is reasonable to conclude that most cases of vaccinal tetanus are *secondary wound infections*, and therefore largely preventable.

2. *Syphilis*.—With the use, years ago, of humanized virus, and particularly in the days of arm-to-arm vaccination, extremely rare instances of the transmission of syphilis have been known to occur. Since, however, the use of calf virus, which is the virus exclusively employed in this country, such an accident is absolutely impossible, as calves are not susceptible to luetic disease.

3. Cancer, foot-and-mouth disease, tuberculosis, and various chronic skin eruptions have been attributed to vaccination by its opponents; none of these claims has, however, been substantiated.

Protective Value of Vaccination.—Of the value of the protection afforded by vaccination against smallpox there can be no doubt in the minds of right-thinking and unbiased persons. The history of the world before the days of universal vaccination shows the wide prevalence of smallpox and its fearful mortality. It was regarded as a disease of childhood, owing to the fact that all contracted it at the earliest opportunity, and, accordingly, smallpox was the cause of a fearful infant mortality.

At the present day, owing to the general employment of vaccination, smallpox is a rare disease, but its very rarity has fostered a certain degree of false security and carelessness in carrying out the process. A young and new generation of non-vaccinated persons in any community is a source of danger, and, accordingly, sporadic cases are often blessings in disguise, from the fact that, when they appear, compulsory vaccination is then instituted and large numbers seek revaccination.

In Germany, where vaccination is compulsory, smallpox is now a comparatively rare disease. While the general death-rate from all diseases is lower in England and Wales than in Germany, the smallpox mortality is seven and one-half times the mortality of Germany, and, proportionate to the population, over thirteen times.

Austria, one of Germany's neighbors, had, for the twenty years following 1874, almost thirty times as high a smallpox mortality as Germany. During this period 239,800 persons perished in Austria from smallpox alone.

Physicians should carefully impress upon those over whom they have any influence the necessity of being vaccinated, for only a thoroughly vaccinated population can solve the problem of exterminating smallpox as an epidemic disease.

Unfortunately the propaganda of the antivaccinationists is capable of doing much harm. In 1921 the Public Health Service¹ reported more than

¹ Public Health Rep., 1921, 36, 2555.

16,000 cases of smallpox in eight states alone in which histories were furnished in 1920; while, from information supplied by only seven states, more than 18,000 cases have been reported with history during the first six months of the present year. In Minnesota, for example, 8238 of these smallpox cases were actually reported. The real lesson, however, is to be found in the statistics of vaccination for this formidable array of patients. More than two-thirds of the entire 34,000 afflicted had never been successfully vaccinated. About one-twentieth of them had been vaccinated more than seven years before the attack; and of the remainder, the histories were in most cases uncertain with reference to their vaccination status, or were not obtained. Only 2 per cent. of the patients were actually reported to have been vaccinated within seven years prior to the attack. Again the lesson to the public is clear and imperative—and there is little left for the consolation of the antivaccination cult, which is doubtless responsible directly or indirectly for some of this unnecessary suffering.

For those seeking a clear, concise, scientific, and calm statement of facts bearing upon vaccination, I can recommend the pamphlet prepared by Schamberg¹ on "Vaccination and Its Relation to Animal Experimentation," and not a few physicians who have seen the ravages of smallpox are ready to shoulder the challenge of the late Osler:

"I would like to issue a Mount Carmel-like challenge to any ten unvaccinated priests of Baal. I will go into the next severe epidemic with ten selected vaccinated persons and ten selected unvaccinated persons. I should prefer to choose the latter—three members of Parliament, three antivaccination doctors, if they could be found, and four antivaccination propagandists. And I will make this promise, neither to jeer nor to gibe when they catch the disease, but to look after them as brothers, and for the four or five who are certain to die I will try to arrange the funerals with all the pomp and ceremony of an antivaccination demonstration."

VACCINATION AGAINST RABIES

There are but few diseases more dreaded by the laity than rabies, or hydrophobia. Tales of the sufferings of infected persons, especially those with the *furious variety* of this infection, characterized by maniacal symptoms and dread of water (hydrophobia), have been thoroughly disseminated, so that the cry of "mad dog!" on the public streets is sufficient to arouse a general state of hysteric excitement in which an otherwise harmless creature may be compelled to bite or snap for self-protection. Not all dogs under these conditions are mad or infected with rabies, and the bite of an angry dog, otherwise normal, is not necessarily dangerous from the standpoint of rabic infection. However, almost every one, upon being bitten by a dog, will promptly consult his physician, and this is proper and to be encouraged. Genuine rabies is an acute infectious disease in which the diagnosis is quite readily made, and whenever possible an effort should be made either to confirm or to disprove the diagnosis by making an examination of the animal's brain, and if the dog is found to have been free from rabies, this fact should be carefully impressed upon the patient, as otherwise the dread of infection may weigh heavily upon the patient and lead to distressing nervous disturbances.

Etiology of Rabies.—While the infectiousness of rabies has been known for a great many years and was proved experimentally by Galateir² and

¹ Published by the Amer. Med. Assoc., 535 Dearborn Ave., Chicago.

² Compt. rend. Acad. d. sc., 1879, lxxxix, 444.

Pasteur,¹ it was not until Negri, in 1903, described certain bodies (Negri bodies), seen by him in large nerve-cells in sections of the central nervous system, that anything was found that seemed absolutely specific for rabies. Negri regarded these bodies as specific for rabies and probably of a protozoan nature. Later investigations fully established the diagnostic value of these bodies, and their definite characteristic morphology, evidences of cyclic development, and staining qualities indicate a protozoan structure resembling members of the Rhizopoda, designated by Anna Williams in 1906² as *Neurorhynchus hydrophobiae*.

Rembringer,³ Poor and Steinhardt,⁴ Bertarelli and Volpino⁵ have demonstrated the filterability of the rabic virus, and Noguchi⁶ has cultivated from both "street" and "fixed" virus very minute granular and somewhat coarser pleomorphic chromatoid bodies which, on subsequent transplantation, reappeared in the new cultures through many generations and reproduced typical symptoms of rabies in dogs, rabbits, and guinea-pigs.

The virus or parasite is contained in the saliva of the rabid animal, and infection is possible when the skin is abraded by bites and scratches. The virus travels by way of the nerve-paths to the central nervous tissue, and, as in tetanus, the symptoms of the disease are due to involvement of these tissues.

Susceptible Animals.—A large number of animals including human beings are susceptible to rabies. In most countries dogs constitute the highest percentage because of their large numbers and freedom with consequent chances of being bitten and infected. Cats, horses, cattle, sheep, goats, hogs, chickens, and animals of prey, as wolves, foxes, badgers, prairie dogs, and martens also contract rabies when bitten by rabid animals, and behave quite similarly to rabid dogs, cattle, and cats.

Immunity in Rabies.—Rabies is a disease that may attack a large variety of the lower animals as well as man. The disease has occurred in practically all of the domestic animals including chickens. The lower animals as well as man possess no demonstrable degree of natural immunity when the virus is introduced in bites. The lower animals, as rabbits and dogs, may prove refractory to infection when the virus is injected intravenously, but are readily infected by intra-cerebral and intra-ocular inoculations. Once the virus has been introduced in sufficient amounts in bites, the disease is invariably fatal in the lower animals. Whether or not human beings inoculated with virus in bites ever escape the disease in the absence of local destruction of the virus in the wound or by vaccination, is not definitely known, but the indications are that human beings possess little or no natural immunity and are very liable to develop the disease when actually infected.

After infection has occurred, the disease is invariably fatal in both human beings and the lower animals. Our body cells do not appear able to produce protective or immunity principles quickly enough to destroy the virus unless they are hyperstimulated by injections of vaccine, as in Pasteur's method of prophylactic vaccination.

Skepticism Regarding Rabies.—While the exact etiology of rabies is unknown, the germ probably being in the Negri bodies, there can be no reasonable doubt of the existence of the disease in the lower animals and human beings. The disease, however, is not one of human beings, but of

¹ Compt. rend. Acad. d. sc., 1881, xcii, 159.

² Proc. New York Path. Soc., 1906, vi, 77.

³ Ann. de l'Inst. Pasteur, 1903, xvii, 834; 1904, xviii, 150.

⁴ Jour. Infect. Dis., 1913, xii, 202.

⁵ Centralbl. f. Bakt., Orig., 1904, xxxvii, 51. Bertarelli *ibid*.

⁶ Jour. Exper. Med., 1913, xviii, 314.

the lower animals transmissible to human beings. There are many persons, including physicians, who doubt the existence of rabies in human beings and ascribe the symptoms of so-called cases the result of hysteria and fright. The author has seen several cases in human beings—all fatal and Negri bodies found in the brains of all—presenting such a terrific and pitiable picture of suffering before death, that once seen the disease is never forgotten and leaves no room for doubt.

Discovery of Vaccination.—To Pasteur is due the credit for having discovered (1880) the fact that the disease may be prevented by conferring gradual immunization with increasing doses of the attenuated virus. This treatment, with some modification, is now used with evident success in all parts of the world.

In 1885 Pasteur¹ first demonstrated the protective value of vaccination against rabies in dogs by means of the injection of emulsions of the dried spinal cord and medulla oblongata of rabid rabbits. These experiments were very successful and in a letter Pasteur stated: "I have not yet dared to treat human beings after bites from rabid dogs; but the time is not far off, and I am much inclined to begin by myself—inoculating myself with rabies, and then arresting the consequences; for I am beginning to feel very sure of my results."

Finally on Monday, July 6, 1885, there was brought to Pasteur, a little Alsatian boy, Joseph Meister, and a man, Theodore Vone, who had been bitten by a rabid dog. The man had been bitten on the arm and Pasteur noting that his clothes had wiped off the dog's saliva and that his shirt-sleeve was intact, reassured him and sent him back to Alsace. But the boy had been severely bitten and Pasteur experiencing great emotion at the sight of the fourteen wounds, finally consented to try vaccination after being encouraged to do so by his friends Vulpian and Grancher. The substance chosen for the first dose was a fourteen-day cord, the injection being made two and half days after the bites. Twelve injections were given, the final one being on July 16th, with some medulla only one day old and fatal for rabbits in seven days; the following days were indeed anxious ones for Pasteur, but nothing developed and the child returned home well and happy. On October 14th, of the same year, a shepherd lad, Jupille, had been severely bitten by a rabid dog while courageously defending a group of children; six days later Pasteur commenced the treatment which likewise proved successful and induced the great discoverer to establish a "service" in Paris for these inoculations. On November 9th he was induced to treat a little girl of ten years who had been severely bitten on the head thirty-seven days previously. Pasteur surmized that it was too late for vaccination, but reluctant to stand by without an attempt, gave a few injections. True to his prediction, rabies developed with a fatal result which affected Pasteur in a profound manner.

Incubation Period of Rabies.—The period of incubation in dogs, or the time elapsing between the time of injury and the first symptoms, is quite variable, ranging from fourteen to sixty days, although it may be as short as ten days. As in tetanus, this period depends upon—(a) the location of the injury; (b) the quantity or dose of virus; (c) the kind of animal responsible for the injury. Bites about the face and fingers, especially if they are deep and lacerating, are especially dangerous; bites about the back and lower limbs, especially if superficial, are much less dangerous, and accompanied by a longer period of incubation. It is to be remembered that bites may be infectious as early as nine days before the dog shows well-marked symptoms

¹ See the *Life of Pasteur* by Mrs. Devonshire, Doubleday, Page & Company, New York.

of the disease. Not infrequently an animal is observed to be surly and snappy for several days before rabid symptoms develop, and a bite during this time should be regarded as dangerous.

Reichel¹ has given the period of incubation of rabies in the domestic animals, including man, as follows:

Man, fourteen to ninety days.

Dogs, fourteen to sixty days.

Cats, fourteen to sixty days.

Cows, fourteen to eighty days.

Horses, twenty-one to ninety days.

Swine, sheep, and goats, twenty-one to sixty days.

Birds, fourteen to sixty days.

Rabbits, nine to ninety days.

Guinea-pigs, eight to sixty days.

It is an exceedingly rare occurrence for man or any of the lower animals to develop rabies following the one hundredth day from the time of exposure or infection, but there are rare instances in which exceptionally long periods of incubation have been observed.

Only about 16 per cent. of human beings bitten by rabid animals and untreated appear to contract rabies. Since the establishment of the Pasteur treatment of the disease the percentage of developed cases after bites is much lower—about 0.46 per cent.

Physicians may be called upon to diagnose rabies among the lower animals in relation to bites of human beings; the main symptoms in the dog and cat are as follows:

Symptoms of Rabies in the Dog and Cat.—Two forms or stages are usually recognized—the furious or irritable and the paralytic or dumb. The furious stage is usually followed by the paralytic stage; but not infrequently the paralytic stage may follow the premonitory symptoms without the furious stage.

First a decided *change in the disposition of the dog* is observed. The animal becomes increasingly restless. There is a *gradual and progressive paralysis of the muscles of the pharynx* which later extends to the jaw and tongue. As a result there is great thirst with *strenuous attempts to swallow* and an *increased flow of saliva* which is whipped into froth. *The appetite becomes depraved* with attempts to swallow straw and dirt; a *change occurs in the bark*. If free, the dog will start out and travel long distances, usually returning greatly changed in appearance. *The hind legs become paralyzed* and the paralysis gradually extends forward, initiating the paralytic stage, which ends in death. The whole course of the disease is from two to eight days.

Or the paralytic stage may follow the premonitory symptoms at once. The animal seeks a secluded spot and is rapidly overtaken with drowsiness. The paralysis of the muscles of the pharynx and face rapidly progresses and death usually follows in two to three days.

The *cat* generally hides in some dark place where it may die in a day or two unobserved. As a rule, however, there is danger for human beings and especially children, the animal jumping up to the face and inflicting severe wounds with its teeth and claws and biting itself. The voice is lost, the animal mewing hoarsely; the appetite is lost, there is rapid emaciation, thirst, and progressive paralysis, ending in death in a few days.

Diagnosis and Management of Rabies.—The bite of an infected animal may give the disease before the animal shows symptoms. Fifteen days is

¹ Amer. Vet. Rev., January, 1911.

the longest time recorded between a bite and the appearance of symptoms in the dog. Therefore, if an animal is kept under observation at least three weeks without developing symptoms, he may be pronounced free from suspicion.

In case of bites by an animal suspected as infected with rabies an effort should be made to quarantine the animal for a sufficient period to determine whether or not rabies is present. If he is killed at a time when there are none or indefinite symptoms the diagnosis may be always in doubt because Negri bodies may not be found in the brain at this time. It is probably very rare, indeed, for an animal to recover from a slight attack of rabies; usually the disease progresses to a fatal termination.

Local Treatment.—As a general rule, all animal bites should receive surgical attention. Wounds produced by animals clinically rabid should be cauterized at once with fuming nitric acid or pure phenol. This is done to offset the delay in securing the Pasteur treatment, and because there is evidence to show that thorough cauterization of the wound is in itself highly beneficial.

Disposition of the Animal.—The animal should be promptly destroyed if it is unmistakably rabid not only to prevent further damage, but in order to make a microscopic diagnosis by examination of the brain for Negri bodies. In destroying the animal care must be exercised not to injure the brain. This examination is highly important and should never be omitted, for if it shows the absence of bodies, this fact should be carefully impressed upon the patient, as there is no doubt that a neurotic element, amounting in many instances to actual hysteria, may cause considerable harm to the patient even though he is definitely free from rabid infection.

The whole dog may be packed in ice and shipped at once to a central laboratory, or the head alone may be cut off close to the shoulders and packed in ice or equal parts of water and glycerin and promptly shipped. The brain should not be disturbed. When it reaches the laboratory the diagnosis should be made at once by "smear" preparations and sections of the brain demonstrating the characteristic Negri bodies in the large ganglion-cells, and confirmed by inoculating emulsions of the brain into guinea-pigs or rabbits. The latter requires from ten to twenty days before the result may be known. A negative animal inoculation test is better evidence than a negative smear or section; obviously, these examinations are to be made only by properly trained persons.

If the animal is not manifesting symptoms of rabies, do not kill; to do so may leave the diagnosis in doubt. The animal should be confined in quarantine for at least three weeks. If infected with rabies it will surely die within this period. If, on the contrary, he remains well throughout this period, he was not rabid and the wound was not infected with rabies virus. Dogs infected with rabies rarely recover; there are no "light" cases with recovery.

Diagnostic Value of Negri Bodies.—According to Park, the value of the smear method of diagnosis may be summarized as follows:

1. Negri bodies demonstrated, diagnosis is rabies.
2. Negri bodies and suspicious bodies not demonstrated in fresh brains, not rabies.
3. Negri bodies not demonstrated in decomposing brains, uncertain.
4. Suspicious bodies in fresh brains, probably rabies.

Indications for Vaccination.—If the animal was clinically rabid, the Pasteur treatment should be commenced as soon as possible, without waiting for the laboratory report, if this will be delayed for several days. Wounds about the face and hands, where there is no clothing to retain the infectious

saliva, should receive intensive treatment; otherwise the milder course of immunization will suffice.

Formerly it was a general rule to send the patient to a regular Pasteur institute, where there are special facilities for the proper treatment of these cases. At the present time, however, *the physician may treat the patient at home*. Several large manufacturing firms are prepared to ship by mail the fresh daily treatments properly preserved and ready for administration.

The Pasteur treatment should be given to every patient bitten by a rabid animal or by one suspected of being rabid. Not all persons are necessarily infected, even by bites of rabid animals, but this should not unduly influence the physician, for he will not have fulfilled his duty unless he carefully explains the etiology of the disease and advises immediate immunization. Aside from the actual benefits of the treatment, the mental effect upon the patient is deserving of consideration. Even slight wounds by rabid animals, wherever their location, should be regarded as dangerous, and the Pasteur treatment advised in addition to routine cauterization.

With severe bites of angry but not necessarily clinically rabid dogs, the treatment depends upon various factors. In any case the wound should be thoroughly cauterized and the animal carefully guarded (not killed) for two or three weeks. If rabid symptoms appear, the animal should be destroyed, the cerebral tissues examined, and the Pasteur treatment of the patient begun.

Disposition of Bitten Animals.—All animals bitten by a rabid animal should, of course, be promptly destroyed; even those bitten by a dog suspected of being rabid should be destroyed or closely guarded until a definite diagnosis can be reached. In England, where strict laws are enforced relative to the muzzling and control of dogs, rabies is relatively infrequent, and it is especially urged that similar measures be adopted and enforced in our own communities, particularly during the summer months. Valuable dogs may receive one or two doses of vaccine and quarantine for at least six weeks; this method of vaccination of dogs, however, is still in the experimental stage and great care is required.

Principle of the Pasteur Treatment (Active Immunization) of Rabies.

—This method is based upon the principle of stimulating the production of rabic antibodies by injecting attenuated or modified virus during the period of incubation, so that the virus introduced into the wound is destroyed, neutralized, or its effects neutralized, while the virus itself is finally destroyed. Pasteur worked out this theory and established its truth by experiments upon the lower animals before applying the treatment to man.

By passing the virus through a series of rabbits the period of incubation is shortened to about six to seven days, and at the same time its pathogenicity for man is actually diminished (*virus fixé*). By drying the tissues containing the fixed virus attenuation is secured, so that it is easily possible so to modify the virus that it cannot produce rabies in man, but yet is able to produce the specific antibodies. The Pasteur treatment is, therefore, a process of active immunization with emulsions of a tissue (spinal cords of infected rabbits) in which the virus has been attenuated by a process of drying and desiccation. The early doses consist of highly attenuated cords, and succeeding doses become gradually more potent, as is usual in the technic of any method of active immunization.

After the adoption of Pasteur's method, modifications in the preparation of this vaccine soon appeared. Hogenes,¹ for example, claimed that Pasteur's method of drying spinal cords did not modify the virus, but simply de-

¹ Nothnagel's Spec. Path. u. Therap., 1897.

stroyed it and that the same result could be obtained by using dilutions of fresh cord. Ferran has likewise used unchanged fixed virus and in this country the method has been advocated by Proeschel.¹ Other methods of attenuating fixed virus have been used, such as exposure to the action of heat, cold, gastric juice, glycerin, and phenol.

Mixed Serum and Vaccine Immunization of Rabies.—Fermi,² in Italy, has advocated immunization with mixtures of antiserum and vaccine. The vaccine is a 5 per cent. emulsion of the most virulent fixed virus made from the brain of a rabbit or dog and rendered avirulent with 1 per cent. phenol. The antiserum is prepared by immunization of the horse. One part of serum is mixed with 3 parts of vaccine, allowed to stand on ice for twenty-four hours, and 3 c.c. injected subcutaneously in the morning and 3 c.c. in the evening. This is repeated for five or ten days, and then

vaccine alone is injected for an additional twenty or fifteen days. Similar serum-vaccine mixtures have been employed by Marie, Remlinger, and Babes.

Contraindications to Vaccination.—There are practically no contraindications. Some individuals are more apt than others, however, to exhibit reactions of hypersusceptibility to the injections. Pregnant and nursing women may be immunized with safety.

Pasteur's Method of Preparing Rabies Vaccine.

—As a preliminary, it is necessary to prepare or obtain *virus fixé*. This may generally be procured from a laboratory, or may be prepared by passing street virus from the medulla of a rabid cow or dog through a series of young rabbits. After from 30 to 50 passages the incubation period is gradually reduced to six or eight days (*virus fixé*).

1. From an animal succumbing the day or night before, a piece of the floor of the fourth ventricle measuring about 2 cm. in length is emulsified in 1 c.c. of sterile bouillon, and 3 or 4 drops of this emulsion are injected beneath the dura of a normal rabbit. In large institutes two or more rabbits are injected daily. The inoculation is quickly and easily performed by trephining a small area in the median line of the forehead and injecting the emulsion beneath the dura mater with a syringe. The whole operation must be carried out in an aseptic and practically painless manner.

2. After inoculation the animals are placed in clean cages; in from six to eight days paralytic symptoms of rabies appear, followed in three to four days by death. The hair is then sprayed with a solution of lysol and the skin removed. The cord and brain are then extracted under aseptic precautions. The cord is severed just below the medulla, a portion is snipped off into sterile bouillon for culture, and then divided into two equal pieces which are suspended by sterilized silk threads in a sterile glass jar containing flakes of caustic potash (Fig. 179). The medulla is placed in a sterile dish, and is used to continue the inoculations, as was previously described. A



FIG. 179.—PREPARATION OF RABIES VACCINE.

Note the cords suspended within the jar by means of sterile silk threads; sticks of sodium hydroxid to absorb moisture and hasten desiccation.

¹ Arch. Int. Med., 1911, 8, 351.

² Supplement to Ann. d. l'Igiene, 1916-17, 26.

postmortem examination is finally performed, and any cord in which the animal is found diseased or in which the culture of the cord shows bacterial contamination is rejected.

The jars with suspended cords are kept in a special room at a temperature of about 20° to 25° C.

3. After a suitable period of drying pieces of cord are prepared for injection. This is performed in various ways at different laboratories; no attempt at exact dosage is made. In the New York Board of Health laboratories 1 cm. of the cord is thoroughly emulsified in 3 c.c. of sterile saline solution, the process being conducted in an aseptic manner. If the material is to be shipped, an addition of 20 per cent. of glycerin and 0.5 per cent. of phenol is made.

4. After twenty-four hours' drying the cord is known as one-day cord; after two days, two-day cord, etc. Pieces of cord cut off at any time and put into glycerin will retain the same strength for several weeks.

Harris Method of Preparing Rabies Vaccine.—Harris and Shackell¹ have advocated a method of preparing vaccine by drying rabic brain and cord tissues of rabbits in a vacuum at a temperature of -18° C. Harris² later modified this by freezing the material with carbon dioxid snow or liquid air followed by drying in a vacuum and pulverizing. The material is then sealed free from moisture and kept in an ordinary ice-box. By this method one rabbit furnishes sufficient material for the immunization of 20 to 25 patients; it is, therefore, economical and less laborious to prepare than the Pasteur method.

D'Aunoy³ has recently described this method of preparing vaccine and has reported favorably upon its use in the prophylaxis of rabies.

Terrell's Method of Preparing Rabies Vaccine.—Based upon the observations of Cummings that killed virus engendered a greater degree of immunity than the original Pasteur method, with no danger of paralysis or other untoward effects, Terrell⁴ has described the following method of preparing the vaccine or virus sterilized with phenol:

Take 1 gram each of cord and brain, grinding with Wedgewood mortar and pestle, and add, drop by drop, distilled water until there is a thick homogeneous suspension; then add larger amounts until 25 c.c. of distilled water are added, and then add slowly 25 c.c. of distilled water containing 1 gram of phenol crystals. This should be added, a small amount each time, and thoroughly mixed and the process repeated until the 25 c.c. are added, for if added too fast, a curdled-looking specimen results instead of a smooth homogeneous emulsion. Place in incubator and keep there for twenty-four hours at 37° C. This gives a 2 per cent. phenol dilution which will always kill the virus in less than twenty-four hours. At the end of twenty-four hours bring the volume up to 200 c.c. with sterile distilled water, which gives a final dilution of brain and cord, one to one hundred, while the phenol is 5/10 of 1 per cent.

Terrell claims that the same amount of immunity produced with twenty-one doses by the original Pasteur method, can be produced with twelve 2 c.c., doses by this method, while with twenty-one doses by this method there is produced three to four times the amount of immunity obtained with the original method.

Administration of the Vaccine.—Injections are given with a sterile

¹ Jour. Infect. Dis., 1911, 8, 47.

² Jour. Infect. Dis., 1912, 10, 369; 1913, 13, 155.

³ Jour. Infect. Dis., 1921, 29, 261.

⁴ Jour. Oklahoma State Med. Assoc., August, 1920.

syringe. The abdominal region or arm of the patient are bared, a spot touched with tincture of iodine, wiped with alcohol, and the injection given subcutaneously. Keirle does not vary the dose according to the age, both the old and the young receiving the same dose.

Cases of severe injury, such as deep bites about the face and fingers, should be rapidly immunized (*intensive treatment*); in other cases the treatment may be mild (*mild treatment*); the tendency at present is to give all cases the intensive treatment. The uniform dose of cord emulsion, prepared as just described, is 1 to 3 c.c. The series of inoculations may be as follows according to the Hygienic Laboratory method of starting treatment with six-day cord instead of fourteen-day cord as formerly used:

SCHEDULE OF INOCULATIONS IN IMMUNIZATION AGAINST RABIES

DAY.	INTENSIVE TREATMENT.
First (three injections).....	8, 7, 6 day cord
Second (two injections).....	6, 5 day cord
Third (two injections).....	5, 4 day cord
Fourth.....	3 day cord
Fifth.....	3 day cord
Sixth.....	2 day cord
Seventh.....	2 day cord
Eighth.....	1 day cord
Ninth.....	5 day cord
Tenth.....	4 day cord
Eleventh.....	4 day cord
Twelfth.....	3 day cord
Thirteenth.....	3 day cord
Fourteenth.....	2 day cord
Fifteenth.....	2 day cord
Sixteenth.....	4 day cord
Seventeenth.....	3 day cord
Eighteenth.....	2 day cord
Nineteenth.....	3 day cord
Twentieth.....	2 day cord
Twenty-first.....	1 day cord

With the phenol-killed vaccine of Terrell the duration of treatment and the number of doses administered depends upon the severity and site of the injury. In mild cases where the exposure to infection has been through contact of recent wounds with infected saliva, the treatment is one dose daily for twelve days. In cases presenting ordinary wounds of parts relatively free from nerve filaments or remotely located from nerve trunks or centers, the treatment is one dose daily for fifteen days. In case of severe bites on the face and hands, multiple small bites, tooth punctures or scratches of those parts rich in nerve filaments, and deep lacerated wounds of any part of the body, the treatment is one dose daily for twenty-one days.

Reactions.—The injections are usually followed by slight soreness. A reaction of erythema and edema with itching, pain and tenderness, and sometimes with malaise and mild fever may occur; according to Geiger¹ these are especially likely to develop on the seventh and eighth days and again on the fifteenth and sixteenth days of the treatment. These reactions are probably in part traumatic and in part anaphylactic reactions to the injected nervous tissue of the rabbit.

Paralysis and polyneuritis sometimes occur. Neijio² has reported 24 cases in a total of 19,800 persons treated in the Pasteur Institute of Buenos Aires, and in 4 the consequences were fatal. He is inclined to think that the

¹ Jour. Amer. Med. Assoc., 1916, 67, 1518.

² Semana méd., 1917, 24, 10.

paralysis may be prevented by great care in the preparation of the virus and warns against the practice of shortening the course of treatment which may introduce increasing amounts of virus too quickly.

With phenol-killed virus Terrell states that paralyzes do not develop during vaccination.

Results.—According to reliable statistics, the mortality of rabies without the Pasteur vaccination is about 16 to 20 per cent.; with the vaccination the average mortality is about 0.46 per cent. The mortality of those bitten about the face or head is about 1.25 per cent.; of those bitten on the hand, 0.75 per cent.; of those bitten on other parts of the body, a little over 0.25 to 1 per cent. In the Pasteur Institute of Paris only such persons are treated as have been lacerated, so that the virus has gained entry into the wounds. Viala¹ reports that during the year 1915 as many as 654 persons were treated, with a single death. Taking into consideration only those cases in which the diagnosis of rabies has been confirmed in the animal by a competent examiner, the mortality of the cases treated at the Pasteur Institute in Paris for the past ten years, and covering the treatment of nearly 6000 persons, is only 0.6 per cent., which, compared to the average mortality of 16 per cent. without vaccine treatment, speaks most favorably for the value of Pasteur's antirabic immunization.

The bites of wolves are more fatal than those of dogs, the mortality being about 10 per cent. in spite of the intensive treatment, and about 40 to 60 per cent. without treatment.

Terrell states that of more than 550 cases treated with phenol-killed vaccine there have been no cases of rabies.

When symptoms of rabies have appeared, the treatment is unavailing. Antirabic serums have been prepared by immunizing animals, such as sheep and horses, and these should be tried in human patients presenting symptoms, but the results in general have not been uniformly encouraging.

Immunization of Dogs.—Active immunization may be applied to dogs for the direct prevention of rabies as developed by the investigations of Semple² and Umeno and Doi.³ A phenolized vaccine of virus prepared of rabbit brain is commonly employed, a single subcutaneous injection of 5 to 10 c.c. being given. This method has been found satisfactory in Japan where the incidence of rabies is large. The duration of the immunity is not definitely known. The method is recommended for the treatment of all dogs in an infected district; however, after a dog has been bitten by a rabid animal one dose of vaccine is insufficient and it is advisable to destroy the animal. Eichhorn and Lyon⁴ have confirmed the work of Umeno and Doi, finding that one large dose of phenolized fixed virus protects against large doses of street virus.

Duration of Immunity After Vaccination.—There is very little data available on this subject, but the immunity in man following a complete course of vaccine injection probably does not last more than one or two years; the same is probably true of dogs vaccinated against rabies as described above. Therefore, a person bitten by a rabid animal one or more years after immunization should be reimmunized with a complete course of injections.

¹ Ann. de l'Inst. Pasteur, 1916, xxx, 422.

² Memoirs Med. and Sanit. Dept. Gov. India, 1911, N. S., No. 44.

³ Kitasato, Arch. Exp. Med., 1921, 4, No. 2.

⁴ Jour. Amer. Vet. Assoc., 1922, 61, 38.

VACCINATION AGAINST TYPHOID AND PARATYPHOID FEVERS

Our knowledge of vaccination in typhoid fever begins with the work of Pfeiffer and Kolle.¹ These observers, in 1896, immunized two volunteers with heat-killed cultures, and by complete laboratory investigations demonstrated the identity of the immunity following an attack of the disease with the artificial immunity produced by inoculation. At about the same time Wright,² of London, inoculated 2 men with killed cultures, and a year later published the results of the successful vaccination of 17 persons. In 1896 he continued the work in India, where 4000 soldiers were inoculated, with encouraging results. Later, during the Boer War, Wright and Leishman treated 100,000 men, and the results, while good, were not encouraging, due, as pointed out later by Leishman,³ to the fact that the vaccine was damaged during its preparation by overheating. Since 1904 an improved vaccine has been used among the British troops in India in ever-increasing quantities, with uniformly good results.

Antityphoid vaccination was begun in the United States Army in 1908, the vaccine being prepared by Major Frederick F. Russel. Its value has been established so clearly that vaccination is now compulsory. The results obtained in the army have had considerable influence in establishing a wide-spread general confidence in antityphoid inoculation.

Immunity to Typhoid and Paratyphoid Fever.—Typhoid and paratyphoid fever are diseases only of human beings. The anthropoid apes have been infected experimentally by Metchnikoff and Besredka⁴ and Gay and Claypool⁵ have produced a syndrome resembling some phases of typhoid fever in rabbits by the intravenous injection of the bacilli, but these diseases do not occur spontaneously among the lower animals.

Some human beings appear to possess a certain degree of natural immunity; at least there are several instances on record where groups were equally exposed and very probably swallowed about the same amount of infectious material, in which some contracted the disease and others did not. Furthermore, it is well known that some individuals suffer with very light infections suggesting the presence of some natural resistance, while others have little or no evidence of resistance. City dwellers as a group appear to possess more resistance than country folk, who appear to be particularly prone to infection when coming to a city. Vincent⁶ has found that the Arabs possess more natural immunity than foreigners in Algiers, and it is also believed by some observers that the Japanese may possess a certain degree of racial immunity.

One attack of typhoid fever does not protect against subsequent attacks as thoroughly as occurs in the acute exanthemata as smallpox, measles, and scarlet fever. The exact percentage of recurrent attacks cannot be stated because the evidence of the exact nature of the alleged first attack is frequently uncertain, but various observers have stated that in 1 to 3 per cent. of cases of typhoid fever there is a history of a previous attack. Recurrent attacks generally occur late in life (after thirty years of age) and during epidemics, when the bacilli are probably of enhanced virulence, indicating that the immunity conferred by one attack is relative and not absolute.

Persons who have had typhoid fever are still susceptible to the para-

¹ *Deutsch. med. Wchn.*, 1896, xxii, 735.

² *The Lancet*, London, September 19, 1896, 907; *Brit. Med. Jour.*, January 30, 1897, 16.

³ *Jour. Roy. Army Corps*, 1909, 12, 136.

⁴ *Ann. de l'Inst. Pasteur*, 1911, 25, 193.

⁵ *Arch. Int. Med.*, 1913, 12, 613.

⁶ *Ann. de l'Inst. Pasteur*, 1911, 25, 455.

typhoid fevers, although very probably not to the same extent as individuals who have never had typhoid fever.

Various antibodies are to be found in the blood during and for a variable period after typhoid and paratyphoid fever. These are largely agglutinins, bacteriolysins, opsonins, and complement-fixing antibodies. The agglutinins have received most study; they usually disappear within the first year after recovery unless a carrier state supervenes. Posttyphoid and paratyphoid immunity is apparently cellular, that is, the cells remain sensitized and capable of producing antibodies rapidly upon stimulation by reinfection.

Preparation of Typhoid and Paratyphoid Vaccine.—Based upon general principles, the vaccine should be prepared of typhoid bacilli as little changed by heat or chemicals as possible. Russel has prepared the army vaccine with a single avirulent culture which proved by animal experiments and laboratory methods capable of producing large quantities of immune agglutinins and bacteriolysins. As a general rule, however, the vaccine should be polyvalent, and particularly in view of the experiments of Hooker,¹ who has shown by complement-fixation tests consistent antigenic differences among some strains of *Bacillus typhosus*.

The preparation of the vaccine is comparatively simple. The bacilli are grown on agar for twenty-four hours, washed off with sterile normal salt solution, standardized by counting the bacilli, and killed by heating to 56° C. for one hour. As a matter of safety, 0.25 per cent. of tricresol is then added (Russel). The details of the technic are given in the chapter on Bacterial Vaccines. Lipovaccines are no longer employed.

Vaccines should be kept in a cold place and are ordinarily useful for a year, but it is better not to use a vaccine more than four to six months old because of a tendency to loose in antigenic power.

Sensitized Vaccines.—Metchnikoff never fully accepted the belief in the value of heat-killed vaccines, and was actively concerned with vaccines prepared of living bacilli sensitized with their immune serum (sensitized vaccines). Injection of these vaccines into chimpanzees is not followed by any untoward effects, and apparently the bacilli so administered are destroyed at once, as they have not been found in the blood, urine, and feces. Metchnikoff and Besredka² have immunized persons according to these methods and report excellent results. Obviously, there is some reluctance in using a vaccine of living bacilli until extended animal experiments have proved that they are harmless and more efficient than the vaccines of killed bacilli.

At the present time sensitized heat-killed typhoid vaccine has come into use for prophylactic immunization. These were first introduced by Besredka,³ who claimed that they were less toxic than plain vaccines and that the injections were followed by a quicker production of immunity. Cecil⁴ has reported that the sensitized dead vaccine usually produces somewhat less severe local and general reactions and probably gives just as high an immunity. Stoner⁵ found that the reactions produced by sensitized and plain vaccines were about the same, and that the former possessed no distinct advantage over the latter. In my experience sensitized vaccine usually produces somewhat less severe local reactions and probably is absorbed more quickly from the subcutaneous tissues; antibody production (agglu-

¹ *Jour. Immunology*, 1916, 11, 1.

² *Ann. de l'Inst. Pasteur*, 1911, 25, 193, 867; 1913, 27, 597.

³ *Ibid.*, 1902, 16, 918.

⁴ *Amer. Jour. Med. Sci.*, 1918, 155, 781.

⁵ *Jour. Immunology*, 1916, 1, 511.

tinins) is sometimes slightly earlier than engendered by plain vaccines, but the differences are inconstant and irregular.

Gay and Claypool,¹ who have made an extended and extensive study of typhoid immunization, have advocated a polyvalent, sensitized typhoid vaccine sediment for prophylactic immunization against typhoid fever as being superior to other forms of typhoid vaccine. Force² has found that this vaccine produces less reaction, and Sawyer³ has found it more protective than several other types of commercial vaccine. Mayer and Kilgore⁴ found that it produced less agglutinins than plain vaccine, although the production of complement-fixing antibodies was the same. Gay's vaccine consists of the ground sediment of a mixed polyvalent vaccine that has been sensitized by an antityphoid serum and then killed and precipitated by alcohol. From this ground culture the endotoxins are extracted by carbolated saline solution and the remaining sediment of bacterial bodies alone used for prophylaxis and in the treatment of typhoid fever.

For prophylactic purposes 1/10 mg. of dried bacteria, which corresponds to an original bacteria count of about 750,000,000, is administered by subcutaneous injection every other day until three or four doses have been given. This vaccine is claimed to produce a better and more prompt immunity response than other vaccines. It has also been used in the treatment of typhoid fever by intravenous injection.

Method of Inoculation.—The vaccine is best administered at about 4 o'clock in the afternoon, so that the reaction appears during the night and is least likely to be disturbing. It is well to administer a cathartic the day before the inoculation is made. Inoculations should not be given during the menstrual period, as the general reaction is likely to be somewhat severer at this time.

The skin over the insertion of the deltoid muscle is touched with tincture of iodine and the injection given *subcutaneously*. Intramuscular injections should be avoided, as the reactions are more unpleasant and accompanied by unnecessary pain on movement. In making deep injections there is also danger of striking a nerve, a proceeding that may be followed by disagreeable neuritis. Subcutaneous injections in the neighborhood of the clavicle are less likely to be disabling if a local reaction occurs.

After the injection has been given the iodine is wiped away with a pledget of cotton and alcohol; no dressing is necessary.

The syringe and needle should be sterile. Commercial firms have placed the prophylactic on the market in syringes with sterilized needles, accompanied with full instructions as to the technic of administration. *The vaccine should be well shaken before the injection is given.*

When a large number of injections are to be given at one time, a single syringe may be used with a large number of sterile needles, a separate needle being used for each person. The vaccine may be put up in individual ampules or in a stock bottle, the former being preferable. The oral administration of vaccine has proved worthless, although Vaillant⁵ has recently reported apparent successful prophylactic immunization by Besredka's method, consisting of the oral administration of bile followed by killed cultures of typhoid and paratyphoid bacilli by the same route.

¹ Arch. Int. Med., 1913, xii, 613; 1913, xii, 622; 1914, xiii, 471; 1914, xiv, 662; 1914, xiv, 662, 669, and 671; 1916, xvii, 303.

² Amer. Jour. Pub. Health, 1913, iii, 750.

³ Jour. Amer. Med. Assoc., 1915, lxv, 1413.

⁴ Arch. Int. Med., 1917, 19, 293.

⁵ Ann. d. l'Inst. Pasteur, 1922, 36, 149.

Dosage of Typhoid Vaccine.—Three injections are given at intervals of one week. For adults (150 pounds) the doses used in the army have been as follows:

First dose:	500,000,000 bacilli.
Second dose:	1,000,000,000 bacilli.
Third dose:	1,000,000,000 bacilli.

These amounts are contained in 1 c.c. (about 15 minims). Children receive doses in proportion to their weight; if the dose cannot be divided evenly, it is better to give a little more rather than a little less, for children tolerate the injections remarkably well.

Triple Vaccine of Typhoid and Paratyphoid Bacilli.—According to recent experiences in the European armies prophylactic immunization with typhoid vaccine does not afford protection against infections with *B. paratyphosus* A and *B. paratyphosus* B. In this country various investigators have estimated that about 2 to 4 per cent. of the cases of so-called "clinical typhoid fever" are due to paratyphoid infections; apparently the majority of these are with *B. paratyphosus* B. It would appear advisable, therefore, to immunize with these micro-organisms in addition to *Bacillus typhosus*, and particularly members of the Army, Navy, National Guards, and all volunteer organizations called into service in time of war.

At the present time this triple vaccine (T A B) is used almost exclusively. It is usually prepared in such a way that each cubic centimeter contains 1,000,000,000 typhoid bacilli and 500,000,000 of each of the two paratyphoids.

The first dose should be 0.5 c.c. and the second and third doses 1 c.c.; I generally advise a fourth dose of 1 c.c.

Tetravaccine of Typhoid, Paratyphoid, and Cholera Bacilli; Pentavaccine of Typhoid, Paratyphoid, and Cholera Bacilli and Micrococcus Melitensis (Malta Fever).—During the World War Castellani advocated the use of these vaccines as mixtures in order to afford some protection against cholera and Malta fever as well as against typhoid and paratyphoid fevers. In some cases *Bacillus pestis* was also included for immunization against bubonic plague. The vaccines were so prepared that each cubic centimeter contained 500,000,000 typhoid, 250,000,000 paratyphoid A, 250,000,000 paratyphoid B, and 2,000,000,000 cholera. If *Micrococcus melitensis* was incorporated it was added to the extent of 2,000,000,000; *Bacillus pestis*, 500,000,000.

These vaccines were accordingly quite heavy suspensions; the first dose was 0.5 c.c. and subsequent doses 1 c.c. According to Castellani the injections were as well borne as the usual typhoid-paratyphoid vaccine. Castellani and Taylor¹ and Lurie² have reported favorably upon the protective value of these vaccines.

Frequency and Number of Inoculations.—Ordinarily three injections are made at intervals of seven to ten days. If the intervals are less than seven days the immunity may not be so high. Dreyer, Gardner, Gibson, and Walker³ believe that the intervals between doses should be eighteen or twenty days to secure the maximum antibody response.

Contraindications.—The administration of vaccine is frequently followed by some local and constitutional reaction. The later may be accompanied by a temporary lowering of general vitality and resistance. Accordingly, it is possible that individuals with an existing infection, as active tuberculosis,

¹ Brit. Med. Jour., 1917, 2, 356.

² Brit. Med. Jour., 1916, 1, 45.

³ Lancet, April 6, 1918.

may be injured by repeated and exceptionally severe or prolonged constitutional reactions. Discretion should be exercised in this as in other therapeutic procedures. There is no reason to suppose that typhoid vaccine is any more potent for harm than vaccines in general.

(a) In *active tuberculosis* with constitutional symptoms (fever and tachycardia) and loss of weight, typhoid vaccine should not be administered. In arrested and inactive pulmonary tuberculosis the vaccine may be given, but if the individual is below average weight, the doses should be reduced. Baldwin and L'Esperance¹ have observed that tuberculous animals were apparently unharmed by typhoid vaccine. Indeed, the non-specific effects of vaccine upon nutrition and metabolism may prove advantageous. *In the presence of a typhoid epidemic tuberculosis, either active or latent, is not a contraindication to typhoid immunization*, according to Russel and Nichols.²

(b) Under ordinary conditions typhoid vaccine should not be given during acute infections, as tonsillitis, rhinitis, bronchitis, or more severe infections. Medlar,³ however, found that there was no increased susceptibility to streptococci in animals following typhoid vaccination and the main contraindication under these conditions is the possible increased illness of the patient due to added constitutional reaction engendered by the vaccine.

(c) Advanced chronic maladies, as *nephritis* with deficient elimination, and probably *diabetes*, should not be immunized under ordinary conditions.

(d) *Pregnancy* is not a contraindication according to Guerin and his colleagues.⁴ However, it is possible that the reaction may be injurious during the last few weeks of pregnancy, and certainly the vaccine is contraindicated if albuminuria or evidences of pregnancy toxemia are present.

(e) As a general rule it is advisable to omit injections during or just preceding *menstruation* and especially if the history is one of painful menstruation in underweight individuals. Lamb⁵ has found disturbances in menstruation engendered by typhoid vaccine, but none were of much import, and menstruation is not an absolute contraindication under ordinary conditions.

Reactions.—Persons in poor physical condition are more likely than the robust to experience disagreeable after-effects.

The local reaction consists of a red and tender area about the size of the palm of the hand lasting about forty-eight hours. Occasionally the edema and pain may be more marked, but abscess formation is practically unknown.

The general reaction, when present, gives rise to headache, malaise, and sometimes to fever, chills, and occasionally to nausea, vomiting, or diarrhea. The severe reactions are not alarming and disappear quickly.

The inoculated person should abstain from severe exercise for the following twenty-four hours and rest; in the great majority of instances our soldiers have not been inconvenienced and were able to continue with their routine duties.

The following tables, compiled by Russel,⁶ show the proportion of reactions in adults and children:

¹ Jour. Immunology, 1916, 2, 283.

² Jour. Amer. Med. Assoc., 1921, 76, 177.

³ Jour. Amer. Med. Assoc., 1918, 71, 2146.

⁴ Gyn. et Obstet., 1920, 1, 217.

⁵ Arch. Int. Med., 1913, 5, 565.

⁶ Jour. Amer. Med. Assoc., 1913, lx, 344.

PERCENTAGE OF GENERAL REACTIONS IN ADULTS (128,903 DOSES)

Dose.	NONE.	MILD.	MODERATE.	SEVERE.
First.....	68.2	28.9	2.4	0.3
Second.....	71.3	25.7	2.6	0.2
Third.....	78.0	20.3	1.5	0.1

PERCENTAGE OF GENERAL REACTIONS IN 359 CHILDREN, TWO TO SIXTEEN YEARS OF AGE

Dose.	NONE.	MILD.	MODERATE.	SEVERE.
First.....	73.54	24.51	1.67	0.28
Second.....	86.26	11.99	1.75	
Third.....	92.56	0.38	1.06	

A comparison of these tables shows that the general reaction is much more infrequent or milder in children than in adults, even after the first dose; after the second and third doses the difference is more marked.

In former years considerable stress was laid upon the possibility of a negative phase following the inoculation, during which a person was believed to be more susceptible to infection. This is now believed by Leishman, Russel, and others of extended experience to be incorrect, the more general belief being that inoculations may be made and are especially indicated during epidemics of the disease.

Duration and Degree of Typhoid Immunity.—It should be emphasized that immunity following typhoid immunization is not absolute, and an immunized person cannot afford to neglect ordinary precautions against infection. A lowered state of general body health or a large dose of infectious material may at any time result in infection.

The prophylactic treatment should be used in conjunction with well-known sanitary precautions in order to obtain the best results.

The immunity is apparently manifest soon after the first and second doses have been given. The duration is not known definitely. From the rich experience of the British Army in India Colonel Firth¹ concludes that immunity begins to decline in about two and one-half years after inoculation. However, even after four and five years the typhoid rate among the inoculated is, estimated roughly, one-fourth that of unprotected troops.

Revaccination.—Since the duration of immunity is difficult to determine it is advisable to revaccinate whenever typhoid fever breaks out in a community. This is especially true of soldiers, lumbermen, campers, and the like, to whom vaccination may be the sole or most important means of defense. Physicians and nurses constantly exposed should be vaccinated at least every two years and preferably annually. Children may be vaccinated every two or three years, and especially just before going to the country on their summer vacation.

Tests for Immunity.—Agglutinins and other antibodies (bacteriolysins and opsonins) appear in the blood about seven to ten days after the first dose of vaccine and reach their maximum about three weeks after the third dose. After this time they gradually disappear from the serum. But actual immunity persists for longer periods although in diminishing degree. Ty-

¹ Jour. Roy. Army Med. Corps, 1911, xvi, 589.

phoid fever generally confers an immunity for life, but the agglutinins and other antibodies in the serum may disappear within a year of recovery unless the patient is a carrier. Positive typhoidin skin reactions are not acceptable as evidences of immunity, as discussed on p. 712.

The subject may be summarized, therefore, with the statement that when typhoid agglutinins and other antibodies are present in the blood in amounts definitely above normal (1 : 100 or higher) after vaccination, the individual is probably immune to typhoid fever; if the agglutinin titer is 1 : 30 or lower the individual may or may not be immune.

Practical Value of Immunization with Typhoid-paratyphoid Vaccine.—

The value of antityphoid vaccination has been repeatedly and amply demonstrated, and there is no question of its power to prevent infection. This protection is not absolute; typhoid fever has occurred among the vaccinated, but the incidence and mortality are much less than among the unvaccinated.

Vaccination cannot be used as a substitute for sanitary measures. Serious contamination of water and milk supplies at their sources can be checked by sanitary measures, but contamination of water, ice, milk, and food on a smaller scale by typhoid carriers among food handlers and by flies, will continue as sources of infection against which vaccination will afford a large measure of protection.

The value of the typhoid prophylactic therapy is best shown in the army, where conditions are better controlled than is possible in civilian life. In 1911, of a division of United States troops, about 20,000 men along the southern boundary, only 2 cases of typhoid fever developed and both recovered. During this same period of time 49 cases were reported in the city of San Antonio, with 19 deaths. The soldiers mixed freely in the city, ate of fruits and vegetables, drank of the same water, and in this manner were freely exposed, although the sanitary conditions in the camp were excellent.

In 1898, during the Spanish War, there were assembled at Jacksonville, Florida, 10,759 troops, among whom there were certainly 1729 cases of typhoid, and including the suspected cases, this figure reached 2693 cases, with 248 deaths. This camp continued about as long as that in 1911, the climatic conditions and water supplies being practically the same, but the sanitary conditions were bad. The remarkable difference in the typhoid rate cannot, however, be reasonably explained by perfect camp sanitation, and the results in 1911 leave no doubt as to the value of antityphoid vaccination.

The results of vaccination in the Great War have been tabulated by Russel.¹ From April 6, 1917, to November 11, 1918 approximately 4,000,000 men served in the army; during two full years there were 1065 cases of typhoid fever, or one among every 3765 men. In the Spanish War there was 1 among every 7 men (141 per 1000). In 1917 and 1918 the total number of deaths was 156, or 1 death among 25,641 men. In the Spanish War there was 1 death among every 71 men (14 per 1000).

Compulsory vaccination in the army began in 1911; the following table, compiled by Russel, shows the morbidity and mortality rates for a period years preceding and following:

¹ Jour. Amer. Med. Assoc., 1919, 73, 1863.

RATE OF TYPHOID FEVER IN THE ARMY AND IN THE CORRESPONDING AGE GROUP IN CIVIL LIFE FOR THE PAST EIGHTEEN YEARS

YEAR.	ARMY.				CIVIL DEATHS FROM TYPHOID FEVER: AGE GROUP, TWENTY TO TWENTY-NINE YEARS. RATE PER THOUSAND OF POPULATION.	
	Number of cases.	Ratio per thousand.	Deaths.	Ratio per thousand.	Total.	Males.
1900	531	5.75	60	0.43	0.46	
1901	594	9.43	78	0.64	0.42	0.54
1902	565	8.58	69	0.86	0.40	
1903	348	5.82	30	0.28	0.35	
1904	247	5.62	12	0.27	0.33	
1905	193	3.57	17	0.30	0.32	
1906	347	5.66	15	0.28	0.32	
1907	208	3.53	16	0.19	0.28	
1908	215	2.94	21	0.23	0.28	
1909 ¹	173	3.03	16	0.28	0.23	
1910	142	2.32	10	0.16	0.27	0.34
1911 ²	44	0.85	6	0.09	0.23	
1912	18	0.31	3	0.04	0.18	
1913	4	0.04	0.18	
1914	7	0.07	3	0.03	0.15	
1915	8	0.08	0.18	0.17
1916	25	0.23	3	0.03	0.12	0.15
1917	297	0.44	23	0.03	0.11	0.14
1918	768	0.30	133	0.05	0.09	0.11

During the Great War there were 213 deaths from typhoid fever in the United States Army from September 1, 1917 to May 2, 1919; if the conditions of the Civil War had prevailed Russel calculated that the death-rate would have reached 51,133, and under the conditions of the Spanish War, the rate would have reached 68,164.

In France the morbidity was reduced from approximately 7 cases per 1000 men in 1914 to less than 0.1 per 1000 in 1917, when triple vaccination was well under way. Vincent has calculated on the basis of 4,000,000 or 5,000,000 soldiers sent to the French front during the thirty-eight months of the war, that if vaccination had not been practised the number of cases of typhoid would have exceeded 1,000,000 with 145,000 deaths. In all the armies at the front Vincent calculated that vaccination reduced the morbidity at least 7 times and the mortality 8.5 times less numerous than during peace times, and this does not take into account the aggravating influences of the war.

As stated by Russell, it is evident "that antityphoid vaccination, carried out as it was by a personnel which had not been carefully trained in its administration, gave a high degree of protection to our forces under the conditions of hurried mobilization and of warfare, and reduced the rate not only below the rates for previous wars but also below the rate found in civil life in some of the older states where the entire population is protected by all the sanitary measures of modern life."

Russel records that in Hawaii, during September, 1917, there was a water-borne epidemic of typhoid fever of the classic type traced to a Japanese

¹ Voluntary vaccination against typhoid.

² Compulsory vaccination against typhoid.

laborer employed until taken sick in the construction of a water-supply system. The incidence of cases developing among 4085 vaccinated individuals was 13.45 per 1000 and the deaths 0.97; among 812 unvaccinated persons the incidence was 55.41 and the deaths 8.02.

Paratyphoid fever has never been as frequent as typhoid fever and until the outbreak of the World War vaccination in the army was not practised. In 1917 the rate among the vaccinated and unvaccinated was 0.03 per 1000 as compared with a rate of 0.14 per 1000 of population among young men of corresponding age in civil life. In 1918 the rate in the army was 0.05 per 1000, but this was still less than half the civil rate (Russel).

Triple vaccination is now employed almost exclusively in civil practice as well as in the army and navy. Craig,¹ Davison,² Dryer, Gardner, Gibson and Walker,³ and others have shown that the results, so far as can be judged by agglutinin curves, are as good as follows immunization with typhoid vaccine alone, and neither the general nor the local reactions are rendered more severe.

Excellent results have been reported by Spooner, Hachtel and Stoner, and others as to the prophylactic value of typhoid immunization in hospital training-schools for nurses, insane asylums, and other public institutions.

Recommendations.—In view of the satisfactory results obtained in the army, typhoid-paratyphoid vaccination is now obligatory on all members of the army and navy corps. Protection of the individual by immunization is the only measure of protection independent of surroundings and effective under all conditions.

Typhoid-paratyphoid inoculation in civilian practice cannot be as widespread or as readily performed as vaccination against smallpox, as the prophylactic must be administered subcutaneously and more than one dose is necessary.

1. Our various state and city boards of health should endeavor to educate the laity, and, if necessary, offer the prophylactic free of charge in order to build up a vaccinated community as far as this is possible.

2. Persons coming in intimate contact with typhoid patients, such as physicians, nurses, and attendants in hospitals, should be immunized. Hospital authorities are justified in making typhoid-paratyphoid vaccination obligatory on all applicants for admission to training-schools.

3. All inmates of asylums, homes, and other public institutions under forty-five years of age should be immunized and the state should be ready, if necessary, to furnish the vaccine.

4. The physician and nurse should urge vaccination upon all the members of a family when there is a typhoid patient among them, also all persons in a community where typhoid fever is epidemic or threatened.

5. The physician should especially advise immunization of those about to leave their homes for a vacation in some neighboring seashore or mountain resort; also upon travelers visiting small towns and rural districts, persons living in towns with unsafe water-supplies, and persons dependent upon shallow wells for water-supply.

6. In times of epidemics of typhoid fever the physician should urge vaccination and revaccination of all over whom he has influence. Thorough vaccination with proper sanitary conditions offers the best hope of eradicating this dreaded disease.

¹ Jour. Amer. Med. Assoc., 1917, 69, 1000.

² Arch. Int. Med., 1918, 21, 437.

³ Lancet, 1918, 1, 498.

VACCINATION AGAINST TYPHUS FEVER

Immunity in Typhus Fever.—Studies in the immunity of typhus fever are very incomplete owing to the uncertain state of our knowledge regarding the etiology of the disease.¹ The Plotz bacillus, *typhi-exanthematici*, is not now accepted as the primary etiologic agent, although it may be an important organism of secondary infection analogous to the relationship of streptococci to scarlet fever. Wolbach, Todd, and Palfrey, believe that *Rickettsia prowazeki* is the cause of typhus fever transmissible by lice. For some peculiar and unknown reason certain strains of *Bacillus proteus* bear some relationship to the disease on the basis of the development of agglutinins for them in typhus fever (see Weil-Felix reaction).

The disease occurs spontaneously only in man; experimentally it has been successfully transmitted to some of the monkeys and probably to guinea-pigs. There are no evidences of racial immunity—all persons, irrespective of race, age, and sex, appear to be extremely susceptible and especially if the general health and resistance have been undermined by privation, disease, and general misery.

One attack of the disease generally confers an immunity for the balance of life and there is some evidence to the effect that the serum of a recovered individual may prove effective in the treatment of the disease, suggesting the presence of immunity principles in the blood for a short time at least after recovery.

Prophylactic Immunization.—Preliminary studies upon prophylactic immunization of persons with *Bacillus typhi exanthematici*, a Gram-positive pleomorphic bacillus secured in blood-cultures from persons suffering with typhus fever by Plotz, Olitsky, and Baehr,² seem to indicate that a vaccine of this bacillus is capable of reducing the incidence of the disease, although it does not produce an absolute immunity to typhus fever (Plotz, Olitsky, and Baehr³). The vaccine is polyvalent and is sterilized by heating to 58° to 60° C. from half an hour to one hour. The suspension is so diluted that each cubic centimeter contains about 2,000,000,000 bacteria, and is preserved with 0.5 per cent. phenol or tricesol. Three injections, consisting of 0.5, 1, and 1 c.c. respectively, have been given in five- or six-day intervals.

In a subsequent investigation of vaccination with this bacillus carried out in Europe these investigators reported encouraging results.⁴

McCoy and Neill⁵ employed the Plotz bacillus in active immunization of monkeys subsequently infected with typhus virus (Mexican), but with negative results. Schultz⁶ states that there is no conclusive evidence that this bacillus is the cause of typhus fever. Wolbach, Todd, and Palfrey⁷ were unsuccessful in cultivating it from 13 cases in Poland. It may be stated, therefore, that the specific etiologic relationship of the bacillus to typhus fever lacks confirmation, and consequently the prophylactic value of the vaccine is very doubtful.

As previously stated, one attack of typhus fever generally protects for the balance of life. Apparently immunization requires the activity of living virus, as Mollers and Wolff⁸ were unable to vaccinate with heat-killed virus. The results obtained by Mitchell and Richardson⁹ by injections of

¹ The Etiology and Pathology of Typhus, Harvard University Press, 1922.

² Jour. Infect. Dis., 1915, xvii, 1.

³ Jour. Amer. Med. Assoc., 1916, lxxvii, 1597.

⁴ Ibid., 1917, 68, 576.

⁵ Public Health Reports, 1917, June 1, 841.

⁶ Amer. Jour. Med. Sci., 1921, 161, 78.

⁷ Internat. Jour. Public Health, No. 2, September, 1920.

⁸ Deutsch. med. Wchn., 1920, 46, 484.

⁹ Lancet, 1, 742.

1, 2, and 2 c.c. of sterile defibrinated typhus blood are suggestive of successful active immunization, but inconclusive.

VACCINATION AGAINST PNEUMONIA

Interest in the possibility of developing a successful form of vaccination against lobar pneumonia was greatly renewed during the recent war owing to the large number of cases developing in the various camps.

Immunity in Pneumonia and Other Pneumococcus Infections.—Lobar pneumonia is probably the commonest pneumococcus infection in man, and susceptibility varies greatly in different individuals. While many of the lower animals are very susceptible to virulent pneumococci when the organisms are injected into the bronchi or directly into the peritoneal cavity or blood-stream, spontaneous pneumococcus pneumonia among them, comparable to the human disease, is quite uncommon.

Natural immunity to pneumonia is probably largely due to local factors in the respiratory tract. Acute infections, as rhinitis and bronchitis, predispose to pneumonia; likewise sudden and profound chilling of the body and exposure to wet and cold. How these factors act in reducing natural immunity is not definitely known. It may be that phagocytosis by wandering leukocytes on the tracheal and pharyngeal mucosa is interfered with; that the normal expelling activity of the cilia is reduced or that the natural bactericidal activity of the blood is diminished. Rosenow, among others, as a result of blood-culture work, has endeavored to place pneumonia among the bacteremic diseases with secondary localization of the pneumococci in the lungs. Very probably this may occur in some cases. Blake and Cecil, however, have shown in recent studies that the pneumococci invade the tissues at some point or points near the hilus of the lung, spreading subsequently throughout the lobe by way of the interstitial framework and lymphatics. Under these conditions pneumonia is a direct localized disease; patchy or bronchopneumonia is almost certainly a direct infection from the bronchi.

Recovery from pneumonia and other pneumococcus infections is apparently brought about by antibodies in the blood and phagocytosis. Bull has shown the influence of agglutinins gathering pneumococci in the blood-stream in clumps and facilitating their destruction by leukocytes; Cohen and Heist have demonstrated the direct pneumococcidal activity of the whole blood as a factor of natural and acquired resistance, and many investigators have demonstrated the presence of opsonins and their important relation in aiding phagocytosis of virulent pneumococci. In addition to these immunity principles, enzymes derived from the leukocytes and plasma probably aid in the destruction of pneumococci; the products of digestion of the inflammatory exudate may have a similar destructive effect.

It is well established clinically that one attack of pneumonia does not confer lasting immunity against subsequent attacks. Indeed, individuals having had pneumonia are commonly believed to be rendered somewhat more susceptible. For this reason doubt has been entertained regarding the possibility of successful vaccination. Since it has been firmly established that pneumococci are divisible into groups and that the antibodies are highly specific for these groups, it is possible that subsequent attacks of lobar pneumonia in the same individual may be caused by different groups of pneumococci rather than by pneumococci of the same group. At least Cecil and Blake¹ have observed that experimental pneumococcus

¹ *Jour. Exper. Med.*, 1920, 31, 685.

Type I pneumonia in monkeys confers on them an immunity which protects them against subsequent infection with Type I pneumococci, but not against Type IV.

Experimental Vaccination Against Pneumococci.—The injection of the pneumococcus vaccines into rabbits has been found by Alexander,¹ Lister,² Howell,³ Cohen and Heist,⁴ and others to engender the production of specific antibodies. Wright⁵ had previously shown that antibodies may be found after the administration of pneumococcus vaccines to men, and Cecil and Austin⁶ have more recently observed agglutinins and protective bodies in some individuals after the administration of saline vaccines. Cecil and Blake⁷ found that heat-killed vaccines only sometimes engendered agglutinins and protective bodies in the blood of vaccinated monkeys, although active immunity was frequently present in the absence of these demonstrable antibodies, and particularly after vaccination with living vaccines.

These investigations have at least shown that pneumococcus vaccines are capable of engendering the production of protective substances which are probably highly specific for the type of pneumococcus employed. This immunity appears sufficient in the lower animals to prevent pneumococcus bacteremia and to reduce the severity of the pneumonia following the intratracheal injection of virulent pneumococci. Vaccination of monkeys with saline pneumococcus vaccines was found by Cecil and Steffen⁸ to even prevent lobar pneumonia under these severe conditions in which large numbers of living virulent pneumococci are suddenly introduced into the lungs of highly susceptible animals—experimental conditions which do not have a strict analogy to pneumonia in human beings—when three injections of large numbers of pneumococci were given. These investigators⁹ have also reported that the intratracheal inoculation of monkeys with three doses of pneumococcus Type I vaccine renders them completely immune against experimental pneumococcus Type I pneumonia. The mere spraying of the throat with the vaccine, however, did not produce complete immunity, probably because the vaccine was prevented from entering the trachea. The immunity appeared to be cellular in character because little or no protective substances could be demonstrated in the serum of monkeys vaccinated by this method. McCoy, Hasseltine, Wadsworth, and Kirkbride¹⁰ observed that about as many cases of pneumonia developed among human beings inoculated with 1 c.c. of a lipovaccine containing approximately 10,000,000,000 each of Types I, II, and III, as among a control group of unvaccinated individuals. There is, however, apparently sufficient experimental data at hand to encourage and justify vaccination against pneumococcus pneumonia in man.

Kinds of Pneumococcus Vaccines.—Two kinds of vaccines are being employed, namely, a vaccine of pneumococci suspended in saline solution and autolyzed at 37° C. until about 95 per cent. of the organisms have become Gram-negative as prepared by Rosenow,¹¹ and heated saline suspensions. Rosenow regards autolyzed vaccine as less toxic and capable of stimulating antibody production in a prompt manner.

Lipovaccines are not being employed. Wright, Lister and Cecil, and

¹ Jour. Med. Research, 1917, 37, 471.

² Publications of the South African Institute for Medical Research, 1916, No. 8.

³ Jour. Infect. Dis., 1920, 27, 557.

⁶ Ibid., 1912, 16, 665.

⁴ Trans. College Phys. of Phila., 1918.

⁷ Ibid., 1920, 31, 519, 657.

⁵ Jour. Exper. Med., 1918, 28, 19.

⁸ Ibid., 1921, 34, 245.

⁹ Public Health Reports, November 3, 1922, 2735.

¹⁰ Jour. Amer. Med. Assoc., 1922, 79, 1128.

¹¹ Jour. Amer. Med. Assoc., 1918, 70, 759.

Blake have used heated saline suspensions; Cecil has given the following method for their preparation:

"Pneumococci are cultivated from eighteen to twenty-four hours on plain or glucose broth. The culture after killing may be used directly or it may be centrifuged, and the sediment of bacteria be suspended in physiologic sodium chlorid solution. Finally it is heated at 65° C. for one-half hour to kill the pneumococci, and the vaccine standardized by the Wright method or by means of a nephelometer. Cultures are taken to test the sterility of the vaccine, and tricresol is added to a concentration of 0.3 per cent. as a preservative."

Dosage and Administration.—For prophylactic purposes at least three injections should be given at intervals of seven to ten days. Large numbers of pneumococci are required—much larger than of typhoid bacilli for typhoid vaccination.

The vaccine should be polyvalent and prepared of several strains of Types I, II, and III and a large number of strains of Type IV; each cubic centimeter may contain 2,000,000,000 of each of the four types, making 8,000,000,000 in all.

The first dose may be 0.5 c.c. or 1,000,000,000 of each of the four types; the second and third doses may be of 1 c.c. or 2,000,000,000 of each of the four types.

The injections should be subcutaneous and given in the same manner as typhoid vaccine (see p. 794).

Reactions.—Cecil states that "in general it may be said that reactions to pneumococcus vaccine are similar to those following injections of typhoid vaccine. Within twenty-four hours following the injection an area of redness and induration appears at the site of the inoculation, which is usually 2 or 3 cm. in diameter, but may be larger. Occasionally small sterile infiltrations, which disappear spontaneously, follow the injection of large doses. Such reactions appear to be an expression of cutaneous hypersusceptibility.

"The constitutional reaction to pneumococcus vaccine is usually insignificant. In many cases it is entirely absent. In a small percentage of cases, vaccination is followed by headache or backache, general malaise, chilly sensations, and rise in temperature. These symptoms, however, are of short duration."

The contraindications are the same as for typhoid vaccination (p. 795).

Results of Vaccination Against Pneumonia.—Wright¹ employed saline vaccines in South Africa where a severe type of pneumococcus pneumonia has claimed a high morbidity and mortality among the natives of the Rand. He thought that vaccination reduced the incidence of the disease during the first three months following inoculation.

Lister,² working with vaccines of different immunologic types of pneumococci occurring in the Rand, has observed much more encouraging results. Three subcutaneous injections of 1 cc. of a vaccine containing three types and having a total content of 7,000,000,000 of cocci, have rendered a large native mine population absolutely resistant to pneumonic infection by any of these types during the observed experimental period of nine months.

During the Great War pneumococcus vaccine was used extensively in some of the American Army training camps as a prophylactic against lobar pneumonia. At Camp Upton, New York, Cecil and Austin³ vaccinated 12,519 men against pneumococcus Types I, II, and III. Three or four doses

¹ *Drugs and Vaccines in Pneumonia*, 1915, Paul B. Hoeber, New York.

² *Publications of the South African Institute for Medical Research*, 1917, No. 10.

³ *Jour. Exper. Med.*, 1918, 28, 19.

of saline vaccine were given at intervals of five to seven days, with a total dosage of 6,000,000,000 to 9,000,000,000 of Types I and II, and 4,000,000,000 to 6,000,000,000 of Type III. The men were under observation ten weeks after vaccination and during that time no cases of pneumonia of the three types vaccinated against occurred among the men who had received one or more injections of vaccine. In a control of approximately 20,000 men there were 26 cases of pneumonia, Types I, II, and III, during the same period. Altogether there were 17 pneumonias of all types among the vaccinated 40 per cent. of the camp, and 173 pneumonias of all types among the unvaccinated 60 per cent. of the camp.

Following the Camp Upton experiment, Cecil and Vaughan¹ undertook to vaccinate the troops at Camp Wheeler, Ga., against lobar pneumonia, using, in this instance, pneumococcus lipovaccine in place of the saline vaccine which had been employed at Camp Upton. The dosage employed in all cases was 1 c.c. of lipovaccine containing approximately 10,000,000,000 each of pneumococcus Types I, II, and III. Of the entire camp strength, 13,460 men, or about 80 per cent., were vaccinated. The men were under observation from two to three months following vaccination. During this period there were 32 cases of pneumococcus Types I, II and III pneumonia among the vaccinated four-fifths of the camp, and 42 cases of pneumonia of these types among the unvaccinated one-fifth of the camp. The weekly incidence rate for pneumonia of all types among the vaccinated troops was conspicuously lower than that for the unvaccinated troops. It will be seen from these figures, however, that the results of pneumococcus vaccination at Camp Wheeler were not so striking as those obtained at Camp Upton the previous winter. This difference was probably due to several factors, among which might be mentioned the influenza epidemic, which swept over the camp before vaccination against pneumonia had been completed, and the use of a lipovaccine instead of a saline vaccine.

Indications.—Persons susceptible to pneumonia and who suffer with repeated attacks may be protected by vaccination; likewise individuals who are exposed to cold and wet, as industrial workers, firemen, and policemen. Vaccination is certainly indicated in camps and other communities when the spread of pneumonia is especially likely to occur.

VACCINATION AGAINST PLAGUE.

In view of the frightful infectiousness and mortality of plague, prophylactic measures are highly desirable. Extermination of rats and ground squirrels, especially of the former, about the wharves of seaport cities and towns is highly essential, as the disease is transmitted by the fleas of these rodents. Aside from sanitary measures, plague vaccine has now been used extensively, with encouraging results.

Immunity in Plague.—Human beings are very susceptible to infection with *Bacillus pestis*; natural immunity apparently does not exist, and especially during epidemics when the virulence of the bacillus is enhanced and its spread facilitated. Young and old, both sexes, and apparently all races are extremely vulnerable. Of course, not all persons contract the disease during epidemics and when exposed, but their escape is to be ascribed more to the prevention of infection, and especially bites of infected fleas, than to the presence of natural immunity principles. Rats, guinea-pigs, ground squirrels, and monkeys are likewise highly susceptible; mice, adult rabbits, cats, dogs, swine, cows, horses, sheep, and goats generally escape infection, being more

¹ Jour. Exper. Med., 1919, 29, 457.

resistant, but all of these animals may be infected artificially by large doses of virulent bacilli.

Recovery from the bubonic type of plague is generally ascribed to the production and activity of opsonins and phagocytosis, aided by agglutinins, and possibly to a slight extent by bacteriolysins. The pneumonic type is generally fatal. One attack of the disease usually confers a lasting immunity, but second attacks are known to have occurred.

Preparation of Plague Vaccine.—The *Haffkine vaccine* is prepared by growing pure cultures of *Bacillus pestis* in flasks of neutral bouillon to which a few drops of sterile olive oil or butter-fat have been added, to serve as floats from which the surface growth of the bacilli can take place. The flasks are cultivated at from 25° to 30° C. for five to six weeks, and are shaken every two or three days, by which the hanging, stalactite-like colonies are thrown down, so that a new crop of the bacilli can develop in contact with the air.

After growing for six weeks the purity of the culture in each flask is tested by subcultures on agar and by direct smears. The masses of bacilli are broken up by shaking, and the material is sterilized by heating at 65° C. for from one to three hours. Phenol is added to the point of 0.5 per cent., and the fluid is tested for sterility by culture. If it is found sterile, it is finally poured into small vials of from 10 to 30 c.c. capacity.

Kolle prepares a vaccine by cultivating the bacillus for two days in flasks of agar measuring 10 by 9.5 cm. Each surface of agar equals about 15 ordinary agar slant cultures, and an agar slant holds about 15 loopsful of culture (4 mm. loop). A loop of this size holds about 2 mg. of organisms, and, accordingly, a flask of agar contains about 225 loopsful of culture, or 225 doses of 2 mg. each. *Kolle* prepares the vaccine in amounts of 0.5 c.c. per dose (2 mg. of bacilli), and the growths in each flask are removed with 112.5 c.c. of sterile normal salt solution. The emulsion is shaken to break up clumps, heated for one hour at 70° C., and tested for sterility. It is then preserved with phenol or tricresol and placed in ampules containing 0.5 c.c. each.

The German Plague Commission strongly recommended the use of twenty-four- to forty-eight-hour-old agar cultures instead of the old bouillon cultures employed in the preparation of Haffkine's vaccine.

Kolle and *Otto*¹ found attenuated living cultures of pest bacilli more effectual for the immunization of monkeys and other animals than killed vaccines. *Strong*² has likewise employed attenuated cultures in the vaccination of human beings, and found them without danger and more actively immunizing than killed vaccines, although for obvious reasons general immunization with the latter is safer and preferable.

Lustig and *Galeotti* prepare a vaccine of the toxic precipitate produced by dissolving the bacilli in a 1 per cent. solution of caustic soda and neutralizing with 1 per cent. of acetic acid. This precipitate is dried *in vacuo* and redissolved in a weak solution of sodium bicarbonate, the dose for adults being 0.0133 gm. of solid substance.

Terni and *Bandi* inoculate rabbits or guinea-pigs intraperitoneally with the bacillus, and just preceding or directly after death they collect the peritoneal exudate, in which the organisms are allowed to continue growing for twelve hours more. The bacilli are then killed at a low temperature, and the fluid thus obtained, after a preservative has been added, constitutes the vaccine.

¹ Deutsch. med. Wchn., 1903, 29, 493; Ztschr. f. Hyg. u. Infectiousk., 1903, xlv, 507.

² Jour. Med. Research, 1908, 18, 325.

Dosage.—The ordinary dose of Haffkine's prophylactic for adult males is from 3 to 3.5 c.c.; for adult females, from 2 to 2.5 c.c. Haffkine himself has injected larger quantities without resulting harm. He recommends giving a second injection after from eight to ten days. The *injections are given subcutaneously*, with a sterile syringe, into the upper arm or elsewhere in areas where the skin is not tightly bound down.

The **local and constitutional effects** are similar to those in typhoid except that they are slightly intensified. The inoculation is followed by redness and swelling at the seat of inoculation, and general symptoms in the form of rise of temperature and a feeling of illness. The latter pass off in twenty-four hours, but the patient should rest during the first day after inoculation.

Duration of Immunity.—The immunity is apparent a few days after inoculation, but is of short duration. In India the protection is believed to last at least three months and possibly longer. In times of epidemic the inoculations should be repeated at least two or three times a year. The brief duration of the immunity is probably one reason why better results are not secured. Best results are observed during epidemics when protection is afforded for a short time, or until the danger is past. In countries or localities where the disease is endemic, persons may refuse repeated inoculation and thus become susceptible to infection.

Results.—In the pneumonic variety of plague the prophylactic does little or no good, a finding that has also been shown experimentally by Strong and Teague.¹

In the bubonic variety Haffkine's vaccine has in general yielded encouraging results. The protection is not absolute; the immunity is of relatively short duration, and therefore good results are not so readily appreciated when the disease is endemic. The mortality among the inoculated is much lower, *i. e.*, 11 to 41 per cent., as compared with 50 to 92 per cent. among the non-immunized. Haffkine summarized his results a few years ago as follows: Among 186,797 *inoculated* persons there were 3999 attacks, or 1.8 per cent.; among 639,630 *uninoculated* persons there were 49,433 attacks, or 7.7 per cent., with 29,733, or 4.7 per cent. of deaths.

Teague² has recently summarized the results of vaccination with heat-killed vaccines as follows: of 321,621 unvaccinated persons in India, the incidence of plague was 34.4 per 1000 and the case mortality 78.6 per cent.; among 118,148 vaccinated persons the incidence was 7.96 per 1000 and the case mortality 39.5 per cent.

The Indian Plague Commission a few years ago reported as follows:

1. Inoculation sensibly diminishes the incidence of attacks of plague. It is, however, not an absolute protection against the disease.

2. The death-rate is markedly diminished by its means, not only the incidence of the disease but also the fatality being reduced.

3. The protection is not conferred on those inoculated for the first few days after the injection.

4. The duration of the immunity is uncertain, but it seems to last for a number of weeks, if not for months.

After the disease has once developed, vaccination is of no avail. When there is imminent danger of infection, vaccine and antiserum should be given together.

VACCINATION AGAINST CHOLERA

Protective inoculation against cholera was first practised by Ferran, a Spaniard, in 1884, although little definite knowledge as to the value of

¹ Philippine Jour. Sci., 1913, vii.

² Jour. Amer. Med. Assoc., 1921, 76, 243.

the procedure resulted from his work. He is said to have used impure cultures of bacilli isolated from the feces of cholera patients. Broth cultures were prepared, and the *living* organisms injected subcutaneously, using 8 drops for the first and 0.5 c.c. for the second and third doses, the injections being given at intervals of six or eight days. While his method and results have been questioned, he was, however, the first to use a method employed later, with some modifications, by Haffkine in India, with good results.

Immunity in Cholera.—Asiatic cholera occurs spontaneously only in man. Rabbits and guinea-pigs may be infected experimentally by virulent bacilli and appear to suffer most from the endotoxins, but the disease does not occur spontaneously in the lower animals even in cholera districts.

Not all human beings are susceptible; apparently many possess a sufficiently high natural immunity to escape infection. Undernourished, sickly, and elderly individuals are particularly susceptible. The acidity of the gastric juice apparently suffices for the destruction of the bacillus and may explain escape from infection in some instances, but when the bacilli are swallowed in water and food they may escape destruction in the stomach and reach the small intestine where conditions are favorable for growth. In some instances bacilli are found in the feces of carriers without clinical evidences of cholera; either the intestinal epithelium in these cases acts as a barrier or the individual possesses sufficient natural immunity to prevent the development of bacteremia and the pathologic changes of infection.

Recovery from cholera is ascribed to the production of agglutinins and bacteriolysins, both of which are to be found in the serum. Doubtless the opsonins and phagocytosis are also important in this relation. One attack of the disease generally confers an immunity, but this is not absolute and second attacks may occur.

Preparation of Cholera Vaccine.—*Haffkine*, following Pasteur's method with anthrax, uses two vaccines—a weaker and a stronger—living micro-organisms being used in both and injected subcutaneously. Vaccine No. 1 is weaker, and is obtained by growing the bacilli on agar at a temperature of 39° C. Vaccine No. 2 is composed of more virulent organisms, prepared by passing the vibrios through a series of guinea-pigs until a strain is obtained which is invariably fatal to these animals within twelve, or at least twenty-four, hours. Cultures are grown on agar, washed off with 8 c.c. of sterile bouillon or saline solution, and administered in doses of 1 c.c., which is equivalent to about two loopfuls (4 mm.) or 4 mg. of living bacilli.

Kolle has shown, however, both by animal experimentation and in the human being, that heat-killed cultures are equally good, and that living cultures are unnecessary and may be undesirable.

By this method the vaccine is prepared by cultivating a virulent strain on flasks of agar for twenty-four hours, as described in the preparation of plague vaccine, and removing the growths with sufficient salt solution so that 1 c.c. shall contain one loopful (4 mm.) of organisms (2 mg.). The emulsion is then shaken to break up clumps, heated to 53° to 60° C. for from one-half to one hour, cultured to determine sterility, and preserved with 0.5 per cent. phenol.

During the Great War heat-killed vaccines were used exclusively.

Strong has proposed the use of a preparation of the nucleoproteins of the cholera vibrio. Organisms of high immunizing and peptonizing power and of high virulence, are selected for the preparation of the vaccine. One-half of a twenty-four-hour culture on agar, 1 per cent. alkaline to phenolphthalein, is suspended in physiologic saline solution. This suspension contains approximately 30 to 35 mg. of the bacteria. It is heated for one

hour at 60° C., and then incubated from three to four days, after which it is tested for sterility and is then filtered through a Berkefeld filter. The remaining half of the twenty-four-hour agar culture is suspended in saline in the same way as the first portion. The organisms are still living in this portion. The suspension is shaken thoroughly from three to four days. After ascertaining that the growth is pure, this suspension is also filtered through a Berkefeld filter. The two filtrates are then mixed in equal proportion and 0.5 per cent. phenol added. The sterility of each lot of the vaccine is tested by animal inoculation and anaërobically. According to Strong, in prophylactic inoculation the degree of immunity obtained is in proportion to the amount of immunizing substances injected. The irritating substances of the cholera vibrios having been removed in the course of the preparation of the vaccine by this method, doses equivalent to many times the usual prophylactic dose of bacterial suspensions may be employed without severe local or general reaction. The adult dose recommended by Strong is 2 c.c., injected subcutaneously. Only a single dose is given.

Dosage.—Kolle's vaccine is given subcutaneously in two injections about a week apart—1 c.c. the first time and 2 c.c. the second time.

As a general rule the heat-killed vaccines are made up to contain about 8,000,000 per cubic centimeter. The first dose is 0.5 c.c. and the second dose 1 c.c., a third dose of 1 c.c. is given when possible.

Haffkine's vaccines are given in the same manner at an interval of five days.

The *local effects* are usually marked by more or less pain and edema, which subside in forty-eight hours. The *constitutional effects* are not infrequently severe, being marked by malaise, fever (100°–101° F.), nausea and vomiting, followed the next day in about 10 per cent. of persons by transient diarrhea. Usually all symptoms have disappeared within seventy-two hours.

Results.—Haffkine's prophylactic vaccine has yielded favorable results in India. Powell reports 198 cases of cholera among 6549 non-immunized persons, with a total mortality of 124. Of 5778 inoculated persons, there were 27 cases, with 14 deaths. Much better results were obtained with Kolle's vaccine, and it is now generally used in preference to the Haffkine vaccines.

Murata, during an epidemic in Japan in 1902, vaccinated 77,907 persons. Of these, 47, or 0.06 per cent., developed cholera, and 20, or 0.02 per cent., died. Of 825,287 uninoculated persons, 1152, or 0.13 per cent., died. During a recent epidemic in Russia Franschetti inoculated 11,178 persons. Of these, 8 contracted cholera and 1 died. In St. Petersburg, during 1907–08, 30,000 persons were inoculated. Of these, 12 developed cholera and 4 died. Of 10,000 uninoculated persons, 68 contracted the disease.

During the Great War vaccination against cholera was extensively practised and especially in the Balkans. The results reported by Biuwid,¹ Fornet,² Hueppe,³ Nedrigailoff,⁴ Lüdke,⁵ and others have been uniformly favorable to the extent that the incidence and mortality have been reduced among the vaccinated in cholera infected districts and especially when two injections were given. Savas⁶ reports that among the Grecian troops the incidence of cholera was 42 per 1000 among those inoculated once and 7 per 1000 among those who received two injections, while among the unvaccinated the incidence was 93 per 1000. The mortality among the vaccinated

¹ Wien. klin. Wchn., 1914, 28, 169.

² Deutsch. med. Wchn., 1914, 40, 1681.

³ Berl. klin. Wchn., 1915, 52, 1273.

⁴ Russk. Vrach., 1915, 15, 169.

⁵ Med. Klinik, 1913, No. 43, 1607.

⁶ Wien. klin. Wchn., 1914, 27, 1093.

(twice) was 10.2 per cent. and among the unvaccinated 27.5 per cent. He states that cholera soon disappeared from cholera infected towns after dual vaccinations.

It appears justifiable, therefore, to conclude that vaccination confers some immunity. This protection may be apparent after the first dose, but is more marked about seven days after the second. The immunity conferred is far from being absolute, and it is noteworthy that while the prophylactic diminishes the liability of the inoculated person to cholera, it has less influence on the mortality when the disease occurs in those who have been vaccinated.

Used in conjunction with modern sanitary regulations, however, Kolle's vaccine certainly proves of value and should be used in combating epidemics.

VACCINATION AGAINST DYSENTERY

Immunity in Dysentery.—Both bacillary and amebic dysenteries are diseases apparently confined to human beings. Digestive disturbances and enteritis are predisposing and the disease is most common among young children, old people, and those confined to institutions. During epidemics, however, susceptibility is quite general, probably due to the enhanced virulence of the micro-organism.

During the course of bacillary dysentery agglutinins and bacteriolysins are produced and apparently are mainly concerned in the processes of recovery; phagocytosis, however, is also active and particularly in the intestinal wall.

Antibodies tend to disappear from the blood rather rapidly after recovery, and one attack does not confer a lasting immunity; indeed, the disease tends to become chronic with frequent exacerbations and recurrent infections.

The *bacteriology* of dysentery is discussed in Chapter XL.

Vaccination.—Ever since the discovery of *Bacillus dysenteriae* by Shiga attempts have been made to develop a practical means of vaccination. Owing to the extreme toxicity of the bacilli and the severe local reactions produced by the vaccines, these attempts have usually failed. Shiga¹ injected himself with one-twelfth of a killed agar slant and the local reaction was so severe as to require incision, although agglutinins were produced. Similar local reactions were observed by Kruse² and Rosenthal.³

Shiga⁴ then injected killed cultures and immune serum simultaneously, followed in three to four days by a larger dose of vaccine without serum. He vaccinated 10,000 Japanese in this way, noting a reduction in the mortality; but the immunity lasted only three to four weeks. Vaillard and Dopter⁵ and Dopter⁶ have employed sensitized vaccines and obtained what they thought were encouraging results, but Lüdke⁷ was unable to confirm these observations.

Gay,⁸ Lüdke,⁹ and others have shown that vaccines engender the production of specific antibodies in rabbits, but the problem of an acceptable method for preparing a non-toxic vaccine remains to be solved. Dean and

¹ Centralbl. f. Bakteriöl., 1898, 24, 817, 870, 913.

² Deutsch. med. Wchn., 1900, 26, 637; 1901, 27, 386; 1903, 29, 49.

³ Centralbl. f. Bakteriöl., Ref., 1905, 36, 23.

⁴ Deutsch. med. Wchn., 1903, 29, 327.

⁵ Ann. de l'Inst. Pasteur, 1903, 17, 463.

⁶ Ibid., 1909, 23, 677.

⁷ Die Bazillenruhr, Jena, 1911, 128.

⁸ Univ. Penna. Med. Bull., 1902-03, 15, 307.

⁹ Centralbl. f. Bakteriöl., 1905, 34, 512, 649.

Adamson¹ have proposed a method employing dilute solutions of hypochlorous acid. Vincent² has proposed the use of ether for sterilizing young cultures. Olitsky³ has advocated a lipovaccine in which the bacilli are suspended in a bland oily medium after the method of Le Moignic and Pinoy. This vaccine has been found to produce local reactions, but not of a severe character; antibody production also occurs as the antigen is slowly absorbed.

With a polyvalent vaccine of different types of dysentery bacilli Vincent⁴ has more recently reported encouraging results. In one series the morbidity was 1.6 per cent. as compared with 22.8 per cent. among the unvaccinated. Another series showed among the non-vaccinated 70.57 cases per 1000 with 1.56 deaths; among the vaccinated 8.14 cases per 1000 with no deaths.

VACCINATION AGAINST INFLUENZA

Until the great pandemic of influenza which began in 1915 the medical profession generally accepted the bacillus described by Pfeiffer in 1892 as the causative agent of this infection; while vaccines prepared of micro-organisms recovered from the upper air passages had been advocated by a few physicians for the treatment and prevention of common "colds" and even sporadic cases of influenza, the profession generally had no experience with vaccines in the attempted prophylaxis of the disease in epidemic form.

A large amount of investigation has been devoted to studies in etiology and vaccine prophylaxis with remarkable differences and shifts of opinion and consequent confusion concerning both subjects; the technical difficulties surrounding the isolation and identification of *Bacillus influenzae* and the lack of scientific controls and criteria for evaluating the prophylactic value of vaccines, have been factors partly responsible for this confusion and inconclusiveness. Furthermore, the high incidence and fatal nature of the respiratory complications characterizing the successive waves of the disease that swept the world since 1915 have very much confused the issue as to the primary etiology of the disease itself. However, out of the large amount and skilful bacteriologic work of the last few years it would appear possible and safe to deduce a few conclusions regarding the etiology of influenza as the subject stands today; while the same cannot be said of the subject of vaccine prophylaxis, it would appear that opinions are also beginning to crystallize on this subject.

Immunity in Influenza.—Influenza occurs spontaneously only in man. Young rabbits and guinea-pigs are susceptible to the effects of artificial infection with virulent bacilli (*Bacillus influenzae*, Pfeiffer) and injections of the toxin, but the disease apparently does not occur among the domestic animals even during epidemics. Influenza of horses is regarded as caused by a streptococcus.

Apparently the great majority of human beings are susceptible and especially during the years of later childhood and early adult age. Nursing infants apparently possess some immunity, but it is more probable that they are less exposed to infection. However, during the last great epidemic individual cases of natural resistance were not lacking. Doubtless factors predisposing to infection, as infections of the upper respiratory tract, exert an important rôle.

Whether or not an immunity follows an attack is uncertain. Evidence

¹ Brit. Med. Jour., 1915, 1, 609.

² Jour. State Med., London, 1921, 29, 54.

³ Jour. Exper. Med., 1918, 28, 69.

Compt. rend. Soc. d. biol., 1921, 85, 965.

indicates in my opinion that an immunity of short duration (not over a year) develops in most individuals. On the other hand, some persons are apparently more susceptible, that is, may develop an attack of "grippe" (apparently a light attack of influenza) every year or so, and peculiarly, about the same time each year.

The Etiology of Influenza in Relation to Vaccination.—In the first place a clear distinction should be made between the primary cause of influenza and the secondary complications, notably the pneumonias. The etiology of the latter may be regarded as worked out satisfactorily and caused by one or several of a group of micro-organisms, including *Bacillus influenzae*, different types of pneumococci and streptococci, *Micrococcus catarrhalis*, staphylococci, and *Bacillus Friedländer*. Blake and Cecil¹ have recently produced pneumonias in monkeys with *B. influenzae* similar to those found among persons, and doubtless this bacillus is a frequent cause of pneumonia either alone or in conjunction with one or more of the other micro-organisms mentioned above. Parker² has found that influenza bacilli may produce a toxin, this observation being confirmed by Huntoon and Hannum,³ Albert and Kelman,⁴ Ferry and Houghton,⁵ and believed capable of exerting a pathogenic rôle and especially in the production of the pulmonary lesions. As may be readily expected, the bacteriologic findings in the lesions of the lungs and upper respiratory passages have varied geographically, one micro-organism or a group predominating in one locality and another or group of micro-organisms in a different locality.

These findings, however, by no means prove that any one of these bacteria are the primary cause of influenza. As previously stated, up to 1915 *B. influenzae* was commonly accepted as the cause, although Pfeiffer had not found them in all cases studied in the pandemic of 1892 and the etiologic rôle of the bacillus had not then and is not now definitely established as the primary cause of influenza. During 1916 and 1917 the bacillus was generally found in such a small percentage of cases and pneumococci, streptococci *et al* in such a high percentage, that the rôle of this bacillus as the primary cause of influenza was generally discredited, although in 1919 and within recent months, opinion is again in its favor and bacteriologists have become divided into two main camps, namely, those who accept, or are inclined to accept, *B. influenzae* as the primary cause, as Wolbach,⁶ Opie, Freeman, Blake, Small and Rivers,⁷ Medalia,⁸ Duval and Harris,⁹ Park,¹⁰ Pritchett and Stillman,¹¹ Roos,¹² Huntoon and Hannum,¹³ Small and Stangl,¹⁴ and others, and those who do not, the latter group regarding the germ as absolutely undiscovered or in the nature of a filterable virus as indicated by the researches of Nicolle, and Lebailly,^{15,16} Gibson and Connor,¹⁷ Bradford, Bash-

¹ Jour. Amer. Med. Assoc., 1920, 74, 170.

² Jour. Immunology, 1919, 4, 331.

³ Jour. Immunology, 1919, 4.

⁴ Jour. Infect. Dis., 1919, 25, 433.

⁵ Jour. Immunology, 1919, 4.

⁶ Bull. Johns Hopkins Hosp., 1919, 30, 104.

⁷ Jour. Amer. Med. Assoc., 1919, 72, 556.

⁸ Boston Med. and Surg. Jour., 1919, clxxx, 323.

⁹ Jour. Infect. Dis., 1919, 25.

¹⁰ Jour. Amer. Med. Assoc., 1919, 73, 318.

¹¹ Jour. Exper. Med., 1919, 29, 259.

¹² Jour. Immunology, 1919, 4, 331.

¹³ Jour. Immunology, 1919, 4.

¹⁴ Jour. Amer. Med. Assoc., 1920, 74, 1004.

¹⁵ Compt. rend. Acad. d. sc., 1918, clxvii, 607.

¹⁶ Ann. de l'Inst. Pasteur, 1919, 33, 395.

¹⁷ Brit. Med. Jour., 1918, 2, 645.

ford and Wilson,¹ Yamanouchi et al.,² Olitsky and Gates.³ Many investigators, as Wahl, White and Lyall,⁴ Rosenau⁵ and others have completely failed to produce anything resembling influenza either in man or the lower animals by inoculating pure cultures of *B. influenzae* and filtrates of influenza bacilli and respiratory secretions.

The serum studies of Wilson,⁶ Rappaport,⁷ Kolmer, Trist and Yagle,⁸ Gay and Harris,⁹ Duval and Harris¹⁰ and Fry¹¹ have shown that agglutinins and complement-fixation antibodies may be found in the sera of persons suffering with influenza, streptococci, pneumococci, and other micro-organisms, but these studies prove nothing in regard to the primary etiologic agent and simply indicate that all of these micro-organisms, and notably *B. influenzae*, are at least factors of secondary importance capable of exerting pathogenic changes and eliciting the production of specific antibodies.

At the present time the filtrable organism described by Olitsky and Gates¹² called *Bacterium pneumosintes*, is deserving of consideration as the primary etiologic agent of influenza. These investigators have described changes in the blood and lungs of rabbits and guinea-pigs which follow the intratracheal injection of unfiltered and filtered nasopharyngeal secretions obtained within thirty-six hours after onset, from patients ill with uncomplicated epidemic influenza. Anaërobic cultures of these filtrates have developed a minute bacilloid body, not of the nature of ordinary bacteria and capable of indefinite propagation on artificial media. While the sera of normal persons do not contain agglutinins or precipitins for this organism these antibodies have been found by Olitsky and Gates¹³ in the serum of 17 persons among 19 who were examined from 10 days to five months after recovery from epidemic influenza.

Briefly, therefore, the subject of the etiology of influenza may be summarized as follows:

1. *Bacillus influenzae* has the strongest claim at present for recognition as the primary cause of influenza; if it is definitely proved that this bacillus is not the true and primary cause, but that the primary agent is a filtrable virus, as *Bacterium pneumosintes*, *B. influenzae* will easily fall into the position of first importance as a micro-organism of secondary importance.

2. The complications of influenza and notably the pneumonias may be accepted as caused by *B. influenzae*, pneumococci, streptococci, possibly *Bacterium pneumosintes*, and other micro-organisms either alone or in combination, the bacteriology varying in different localities. These complications, however, are to be separately considered from the etiologic standpoint from the primary cause of the disease.

Kinds of Vaccines.—With regard to the prophylactic value of influenzal vaccine, it is extremely difficult to express an opinion, as is so commonly true of vaccine therapy in general.

Two different kinds of vaccines have been employed, namely, vaccines

¹ Quart. Jour. Med., 1919, 12, 259.

² Lancet, 1919, June 7, 971.

³ Jour. Amer. Med. Assoc., 1920, 74, 1497; *ibid.*, 1921, 76, 640.

⁴ Jour. Infect. Dis., 1919, 25, 419.

⁵ Jour. Amer. Med. Assoc., 1919, 73, 861.

⁶ Lancet, 1919, No. 5014, 607.

⁷ Jour. Amer. Med. Assoc., 1919, 72, 633.

⁸ Jour. Infect. Dis., 1919, 24, 583.

⁹ Jour. Infect. Dis., 1919, 25, 414.

¹⁰ Jour. Infect. Dis., 1919, 25, 384.

¹¹ Lancet, February 14, 1920, 368.

¹² Jour. Exper. Med., 1921, 33, 125, 161, 373.

¹³ *Ibid.*, 1923, 37, 303.

composed of influenza bacilli alone and mixed vaccines of influenza bacilli, pneumococci, streptococci, etc. The latter type has been used more extensively than the former. The mixed vaccines were prepared somewhat as follows:

	Million per cubic centimeter.
<i>Bacillus influenzae</i>	1000
<i>Streptococcus hemolyticus</i>	1000
<i>Streptococcus viridans</i>	1000
<i>Pneumococcus</i> , Types I, II, III, and IV, of each.....	1000
<i>Staphylococcus aureus</i>	500

The first dose was 0.5 c.c.; the second and third doses were 1 c.c. The injections were given by subcutaneous injection at intervals of five to seven days. As a general rule the local and general reactions were of a mild character.

Value of Vaccination.—The value of vaccines has been generally discussed under three headings, namely, their prophylactic value against influenza itself; second, their prophylactic value against the pulmonary complications, and third, their curative value in the treatment of influenza and its complications.

Reports from different parts of the world by Leary,¹ Rosenau,² Paschall,³ Wynn,^{4,5} Minaker and Irvine,⁶ Champstaloup and Drennan,⁷ Cadham,⁸ Bezancon and Legrouse,⁹ Tottenham,¹⁰ Duval and Harris,¹¹ and others indicate that the mixed vaccines have some value in reducing the incidence of the disease and especially the incidence and severity of the complicating pneumonias. Two commissions,¹² composed of Rosenau, Gay and McCoy and Whipple, Davis and Crumm, reported early in 1918, that the available statistical evidence indicated that influenza vaccines may have some prophylactic value, no specific value in treatment, and were probably harmless, but that the whole subject of vaccine therapy required careful scientific investigation for determining its exact status.

Recently Sir William Leishman¹³ has published the following statistical analysis of the use of two mixed vaccines in the British Army:

43,520 non-inoculated soldiers:		
Incidence per 1000.....	47.3	per cent.
Pulmonary lesions.....	13.3	"
Deaths.....	2.25	"
15,624 inoculated:		
Incidence per 1000.....	14.1	per cent.
Pulmonary lesions.....	1.6	"
Deaths.....	0.12	"

McCoy, Murray and Teeter,¹⁴ however, have found no evidence to indicate the prophylactic value of influenzal vaccine and failed to demonstrate

¹ Amer. Jour. Public Health, 1918, 8, 754.

² Jour. Amer. Med. Assoc., 1918, 71, 1602.

³ Jour. Amer. Med. Assoc., 1918, 71, 1602.

⁴ Lancet, 1918, 26, 874.

⁵ Brit. Med. Jour., 1920, February 21, 254.

⁶ Jour. Amer. Med. Assoc., 1919, 72, 847.

⁷ New Zealand Med. Jour., 1919, 1, 63.

⁸ Lancet, 1919, 2, 885.

⁹ Bull. l'acad. d. med., Paris, 1919, 81, 44.

¹⁰ Brit. Med. Jour., 1919, 1, 41.

¹¹ Jour. Infect. Dis., 1919, 25, 384.

¹² Jour. Amer. Med. Assoc., 1918, 71, 1317.

¹³ Lancet, February 14, 1920, 366.

¹⁴ Jour. Amer. Med. Assoc., 1918, 71, 1907.

prophylactic value in properly conducted experiments. Hinton and Kane¹ and others have also reported negative results. Von Sholly and Park² observed that among 1327 inoculated persons and 3025 controls, vaccination with mixed vaccines had very little specific influence either in preventing or causing respiratory diseases other than pneumonia. The difference in the pneumonia incidence, however, was marked, the vaccinated showing an incidence of 0.075 per cent. as against 0.36 per cent. among the unvaccinated. Jordan and Sharp,³ in a study of 2873 persons vaccinated with a mixed vaccine, and 3193 unvaccinated controls, reached the conclusion that any considerable degree of protection against influenza by vaccination is doubtful, but that there was some evidence of prophylaxis against pneumonia.

It would appear, therefore, that the prophylactic value of influenza vaccine is doubtful; however, a general survey of the investigations conducted with *mixed* vaccines rather than vaccines of *Bacillus influenzae* alone does, I believe, permit the following conclusions at the present time:

1. Large doses of mixed vaccines prepared of freshly isolated strains of *B. influenzae*, pneumococci and streptococci confer a slight degree of protection against influenza. It is probable that this immunity is of short duration.

2. These vaccines have decided value in reducing the frequency and severity of the pulmonary complications. While the correctness of the first conclusion is doubtful both from a clinical point of view and especially in the uncertain state of our knowledge concerning the primary cause of influenza, the second may be regarded as well established and a justification for the use and further trial of these vaccines, and preferably under well-controlled conditions, for the purpose of possibly ameliorating the severity and the incidence of epidemic influenza and for securing data of scientific value.

VACCINATION AGAINST THE COMMON COLD

The "common cold" by reason of its tremendous morbidity is of greater importance than commonly considered even though the infection is not of itself of a fatal character. Few people escape at least one "cold" during the winter months and a large number suffer from several attacks each season. Furthermore, the "common cold" may be the starting-point for middle-ear infections, mastoiditis, infections of the accessory nasal sinuses, bronchitis, and pneumonia. It is quite possible that an acute rhinitis may be related to the development of carrier states for meningococci and pneumococci; considered in the light of these possibilities the "common cold" is worthy of very serious investigation, and especially in the direction of its etiology and prophylaxis.

The "common cold," acute coryza or acute rhinitis, is probably a definite entity and not to be confused with true influenza. The so-called "grippe" is intermediate in severity between the "cold" and influenza; the writer believes that the "cold" and "grippe" will be ultimately shown to have the same etiology, the latter being a severer infection, while influenza is caused by a different primary agent.

The Etiology and Immunity.—The etiology of the "common cold" is still unknown. No doubt sudden changes in temperature may induce vascular engorgement of the nasal mucosa with increased glandular secretion which may resemble a "cold" of mild character; it is possible that these changes may favor increased activity of the bacterial flora normally present.

¹ Tenn. State Med. Assoc. Jour., 1919, 11, 442.

² Jour. Immunology, 1921, 6, 103.

³ Jour. Infect. Dis., 1921, 28, 357.

This is one view of the etiology that is widely accepted and bacteriologic studies have shown that streptococci, pneumococci, staphylococci and *M. catarrhalis* are commonly found. Floyd¹ has also found that some patients exhibit cutaneous sensitiveness to these organisms which indicates their close relationship to the disease and opens up the possibility of the "cold" being an expression of clinical allergy to the proteins of these organisms. Krause, Foster, and more recently Bloomfield have expressed the opinion that the primary etiologic agent is in the nature of a filterable virus and unknown. Certainly no one has been able to demonstrate in cultures the presence of an organism that may not be found under normal conditions, and for this reason the streptococci, pneumococci, etc., are regarded as having no relation at all to the disease or but of secondary importance. The writer believes that if it is ultimately proved that an unknown virus is the cause that some of these bacteria will easily fall into a position of important secondary factors; certainly they have been closely identified with the complicating infections, as sinusitis, otitis media, etc.

Little is known of immunity to colds. Some individuals are extremely susceptible; others possess a high degree of resistance. Doubtless, purely local factors are operative in some cases. Whether a transient immunity is conferred by an attack is not known—certainly such resistance is usually very temporary in view of the numerous attacks that may occur during one winter season.

Vaccination.—The question has arisen regarding the possibility and advisability of attempting vaccination against the usual bacterial flora of streptococci, pneumococci, staphylococci, etc., as a means of prophylaxis. A large number of reports indicate that vaccination with vaccines of these bacteria confers a temporary immunity; other reports have been negative. I have had no experience with stock vaccines, but with autogenous vaccines I believe I have seen unmistakable evidences of successful prophylactic immunization. My practice is to make cultures on or about the third to fifth days of the disease during an acute attack and isolate the streptococci, pneumococci, staphylococci and *Micrococcus catarrhalis* that may be present. The bacteria are incorporated into a vaccine in equal proportions so that the final product has a total of 1,000,000,000 bacteria per cubic centimeter. Immediately after the attack has subsided, the vaccine is administered by subcutaneous injection at intervals of five to seven days in doses of 0.1, 0.2, 0.4 0.6, 0.8, and 1 cubic centimeter. In my experience this treatment has protected many individuals for at least one season; in some instances for two years. The vaccine apparently keeps well in a refrigerator for one year, and it is advisable to give three or four injections during the succeeding October and November in an attempt to confer some degree of protection for the following winter.

VACCINATION AGAINST PERTUSSIS

The hemoglobinophilic bacillus described by Bordet and Gengou in 1906 is now commonly accepted as the cause of pertussis or whooping-cough. These investigators² prepared antitendotoxic sera and vaccines and reported that the latter were harmless and provoked no unpleasant symptoms.

Immunity in Pertussis.—Whooping-cough or pertussis occurs spontaneously only in human beings. The lower animals escape infection even when intimately exposed; apes have been experimentally infected.

Children between the first and second dentitions are especially suscep-

¹ Bost. Med. and Surg. Jour., April 15, 1920.

² Ann. de l'Inst. Pasteur, 1906, 20, 731.

tible; nurslings under a year of age may, however, develop the disease. Adults escaping the disease during childhood may become infected, and among these whooping-cough is frequently a serious disease. Negroes are especially susceptible.

Apparently some persons are naturally immune and never contract the disease, even though exposed. It is highly probable, however, that examples of absolute immunity are rare and that some individuals apparently immune have had mild and unrecognized attacks.

One attack of pertussis usually confers an immunity for life. During the disease agglutinins, bacteriolysins, and complement-fixing antibodies develop for the bacillus, but quickly disappear after recovery. Phagocytosis of bacilli in the mucosa of the respiratory tract also occurs and contributes to resistance and recovery.

Vaccination.—In 1914 Hess¹ employed pertussis vaccination and believed that it had protective value in a certain percentage of cases; 26 per cent. of a group of 80 unvaccinated and exposed children did not contract the disease and were probably naturally immune, while 92 per cent. of 244 vaccinated children failed to contract the disease. Luttinger² gave prophylactic injections to 91 children constantly exposed to pertussis for two weeks or less; of these, 94 per cent. escaped the disease. He also vaccinated 277 children who showed early symptoms of the disease; of these, the disease was apparently aborted in about 53 per cent. These results tend to indicate that when the disease is actually present, active immunization is much less likely to prevent progression of the infection than when given before any symptoms have appeared. Luttinger also analyzed the reports of vaccination of 239 children by private physicians, the results indicating that 90 per cent. were apparently protected. Huenekens³ observed the production of complement-fixing antibodies after the administration of vaccine and especially when fresh vaccines were employed. He found prophylactic vaccination effective, but emphasized the necessity for using vaccines not over one week old and in large doses. Bogert⁴ and Luzzati⁵ have also reported favorably upon the prophylactic value of pertussis vaccines; likewise Davies⁶ on both its prophylactic and curative value, but Davison⁷ summarizes the literature with the statement that the evidence in favor of their prophylactic value is slight.

Since the disease is one of the most dreaded of childhood, it would appear that immunization with freshly prepared vaccines is worth while and especially in institutions and families. At least three subcutaneous injections should be given at intervals of five to seven days and as soon as possible after exposure. For children the doses may be 500,000,000, 1,000,000,000 and 2,000,000,000 bacilli respectively; for adults, 1,000,000,000, 2,000,000,000, and 3,000,000,000. The duration of the immunity after vaccination is unknown, but probably is not over two or three years' duration.

VACCINATION AGAINST CEREBROSPINAL MENINGITIS

Immunity in Meningococcus Meningitis.—This disease occurs only in man, although there is considerable evidence to indicate that horses may suffer from a meningitis produced by a diplococcus closely resembling the

¹ Jour. Amer. Med. Assoc., 1914, 63, 1007.

² Amer. Jour. Med. Assoc., 1917, 68, 1461.

³ Amer. Jour. Dis. Child., 1918, 16, 30.

⁴ Amer. Jour. Dis. Child., 1918, 15, 271.

⁵ Policlinico, 1920, 27, 451.

⁶ Amer. Jour. Dis. Child., 1922, 23, 423.

⁷ Jour. Amer. Med. Assoc., 1921, 76, 242.

meningococcus. As far as I know, however, no one has shown that this disease of horses is transmissible to man or the reverse.

Natural immunity of human beings to this infection probably exists to some degree. At least many individuals escape even when intimately exposed, although this may be due to other factors controlling the infection. Children under five years are most susceptible; adults between thirty-five and forty years are least susceptible, although the disease may occur still later in life.

Sex has no influence; neither has race, although negroes have suffered more than whites in some epidemics. This is probably due to hygienic conditions. Catarrhal infections of the upper respiratory tract predispose to infection, doubtless reducing the phagocytic activity of leukocytes and aiding the lymphatic and vascular absorption of meningococci.

Second attacks are rare, but do occur. During the disease agglutinins, bactericidans, opsonins, and complement-fixing antibodies may be found in the blood, but rapidly disappear after recovery. Recovery is apparently brought about largely by the activity of immune opsonins and phagocytosis, aided by agglutinins.

Vaccination.—Sophian and Black¹ have shown experimentally that a polyvalent meningococcic vaccine, heated to 50° C., standardized in the usual manner, and given in three injections in doses of 100,000,000, 500,000,000, and 1,000,000,000, at intervals of a week, appears to afford a high degree of protection. In the blood-serums of inoculated persons these observers were able to demonstrate opsonins, agglutinins, and complement-fixing amboceptors. All evidence points to the efficacy of prophylactic vaccination, as only a moderate degree of immunity may give complete protection against the disease.

Black,² in a subsequent investigation, found the production of antibodies after vaccination and expressed the opinion that the immunity probably lasts for two years. Quarelli³ reports favorably upon this vaccination, and Whitmore, Fennel and Petersen⁴ vaccinated 55 persons with a lipovaccine and observed the production of agglutinins with moderate general reactions after large doses. Gates⁵ administered polyvalent saline vaccine to 3700 volunteers in three subcutaneous injections at weekly intervals of 2,000,000, 4,000,000,000, and 4,000,000,000 or 8,000,000,000 cocci. These doses rarely caused more than the mildest local and general reactions, although exceptionally a more severe reaction with symptoms of meningeal irritation were observed. Specific meningococcus agglutinins were demonstrable in the blood-serum of the vaccinated individuals.

It would appear, therefore, that immunization with polyvalent saline vaccines of meningococci in three doses by subcutaneous injection at weekly intervals may prove of value as aiding in the prophylaxis of meningococcus meningitis and especially when the disease occurs in epidemic form.

VACCINATION AGAINST SCARLET FEVER

Immunity in Scarlet Fever.—Scarlet fever occurs only in human beings. Landsteiner, Levaditi and Prasek⁶ claim to have produced the disease experimentally in the chimpanzee, but not in monkeys; Draper and Handford⁷ also failed to infect monkeys.

Age is a most important factor in susceptibility. According to Welch

¹ Jour. Amer. Med. Assoc., 1912, lix, 527; Black, *ibid.*, 1913, lx, 1289.

² *Ibid.*, 1914, 63, 2126.

³ Policlinico, 1917, 24, 501.

⁴ Jour. Amer. Med. Assoc., 1918, 70, 427.

⁵ Jour. Exper. Med., 1918, 28, 449.

⁶ Ann. de l'Inst. Pasteur, 1911, 25, 754.

⁷ Jour. Exper. Med., 1913, 17, 517.

and Schamberg¹ children from two to five years of age are most susceptible. Considerably over half of the deaths from scarlatina occur in children under five years, almost 90 per cent. under ten and over 95 per cent. under fifteen years of age. Infants under one year are seldom infected and under three months of age the disease is very rare, even though the child suckles a mother with scarlet fever. It is very doubtful if children are ever born with the disease.

Negroes are less susceptible than whites and the mortality among them is much lower. Osler states that the natives of India enjoy a high degree of natural immunity.

Undoubtedly some persons possess natural immunity, but it is not always absolute, as the disease may develop after repeated exposures. The individuals of some families are sometimes peculiarly susceptible, four and five members taking the disease in rapid succession.

The unknown virus very probably gains access through the respiratory tract. One attack usually confers an immunity for life, although second and even third attacks may occur.

Nothing is known of the mechanism of immunity, although immunity principles are evidently present in the blood and the serum of convalescents is sometimes useful in the treatment of the disease. Serum of convalescents has not been used for passive immunization, but it would appear that this may be successful as indicated by the apparent success of this method in the prevention of measles.

Vaccination Against Streptococcus Infection in Scarlet Fever and Puerperal Sepsis.—Several Russian physicians, particularly Gabrickevski,² Longovi, Nitikin, Shamarin, and others, have secured good results from a method of prophylactic vaccination against scarlet fever with a polyvalent vaccine of scarlet fever streptococci. Heat-killed vaccines were given in three successive doses. In this country the method has been tried by Watters³ and Kolmer,⁴ who found that while inoculations with a heat-killed streptococcic vaccine cannot prevent scarlet fever itself, such inoculations may, however, prevent a severe attack of the disease by producing some immunity against secondary streptococcic infections.

Weaver and Boughton,⁵ Weaver and Tunncliffe,⁶ Meakens,⁷ Boughton,⁸ and others have observed the production of specific opsonins and other antibodies after the administration of streptococcus vaccines, and it would appear that active immunization may be of some value in the prophylaxis of streptococcus infections.

Champtaloup⁹ has advised the active immunization of expectant mothers against streptococcus *puerperal sepsis*, when the infection occurs in epidemic form in institutions, or in private practice in which the house surroundings are not of the best. Three doses are given at intervals of two days of 100,000,000, 250,000,000, and 500,000,000, and sensitized vaccines are preferred.

¹ Acute Contagious Diseases, Lea Brothers & Company, Philadelphia and New York, 345. (Unfortunately this valuable book is now out of print.)

² Russk. Vrach, St. Petersburg, 1906, x, 469; see review of literature by Smith in Boston Med. and Surg. Jour., 1910, clxii, 242.

³ Jour. Amer. Med. Assoc., 1912, 58, 546.

⁴ Penna. Med. Jour., February, 1912.

⁵ Jour. Infect. Dis., 1908, 5, 608.

⁶ Jour. Infect. Dis., 1908, 5, 589.

⁷ Jour. Exper. Med., 1909, 11, 815.

⁸ Jour. Infect. Dis., 1910, 7, 99.

⁹ Brit. Med. Jour., 1914, 1, 1221.

VACCINATION AGAINST ACUTE ANTERIOR POLIOMYELITIS

Flexner and Lewis,¹ in 1910, showed that monkeys could be immunized against poliomyelitis by repeated subcutaneous injections of increasing amounts of crude unmodified virus over a period of two and one-half months. These animals, after a further ten days, were injected intracerebrally with 2 c.c. of a filtrate of a highly potent virus, of which from 0.05 to 0.1 c.c. would prove fatal. The immunized animal therefore resisted from twenty to forty fatal doses. In a later report they state that artificial active immunity either by the injection of a single large dose or by a series of increasing small doses of crude virus over a period of time is not uniformly successful. In the former method some of the animals developed poliomyelitis as a result of the subcutaneous injection, and in both some of the animals so injected did not resist the test intracerebral inoculations of the rather large dose of a highly potent virus.

Levaditi and Landsteiner² dried cords from monkey poliomyelitis after the method of Pasteur for rabies, but they found that some of the animals developed poliomyelitis as the result of the preventive inoculations.

Abramson³ employed subcutaneous injections of heat-modified followed by unheated virus for the vaccination of monkeys and found neutralizing principles in the blood of these animals for active virus; five of eight vaccinated animals withstood the intracerebral injection of three to six lethal doses of virus.

These results encourage further investigation but at the present time the method has not been applied to human beings.

VACCINATION AGAINST CHICKENPOX

Immunity in Chickenpox.—Chickenpox, or varicella, occurs only in man; the lower animals enjoy an absolute natural immunity even when intimately exposed to infection.

The disease is most common between one and seven years, but may develop in nursing infants. Adults commonly escape, but those who have not had the disease in childhood are susceptible and may develop the disease.

Both sexes and all races are equally susceptible. Human beings do not appear to have an appreciable degree of natural immunity, although the disease does not attack as many persons as are infected with measles.

One attack usually confers a lasting immunity; second attacks have been recorded, but are exceedingly rare.

Vaccination.—Kling⁴ has reported the successful inoculation of chickenpox followed by protection. The virus should be the clear fluid of vesicles in as early stage as possible both of the disease as well as of the vesicles themselves, and taken, of course, only from children otherwise healthy and free of syphilis and tuberculosis. Kling cleanses a vesicle, inserts the point of a sterile lancet, and makes three to four pricks of the cleansed skin of the arm of the child being vaccinated; three or four more insertions are made of virus from a second vesicle. The mild traumatic reaction subsides in a day or two, followed on the eighth day as a rule by the development of one or more red papules which quickly change to vesicles, followed by scabbing and eventually an insignificant scar. In a small percentage of cases a generalized eruption and slight fever may occur. Of 135 vaccinations,

¹ Jour. Amer. Med. Assoc., 1910, 54, 1780; *ibid.*, 1910, 55, 662.

² Compt. rend. Soc. de biol., 1910, 68, 311.

³ Jour. Amer. Med. Assoc., 1918, 70, 1142.

⁴ Hygiea, 1913, lxxv, 1032; Berl. klin. Wchn., 1913, I, 2083; *ibid.*, 1915, liii, 13.

failures occurred in 23 per cent. These experiments indicate that it is sometimes possible to inoculate and transmit chickenpox by this means and that the resulting infection is usually local.

According to Kling successful inoculation confers immunity, while 73 per cent. of unvaccinated children contract the disease. Handrick,¹ however, was unable to confirm these results during two epidemics with the vaccination of 127 children. In this country Rabinoff² inoculated 142 susceptible children using Kling's method except that scarifications were employed; 114 or about 75 per cent. developed varicella. Of these 6 or 8 per cent. subsequently developed the disease, all being vaccinated within ten days previously and within the incubation period. Rabinoff is convinced that the vaccinations limited the spread of the disease. Michael³ has reported 8 successful inoculations of 32 children by this method.

Hess and Unger⁴ have vaccinated by drawing the fluid of vesicles (not pustules) into sterile capillary tubes to the distance of about an inch, diluting with sterile saline solution, and injecting intravenously. In all, 38 children, three or four years of age, were inoculated by this method; none developed any local or general symptoms or any eruption suggestive of varicella. All were exposed to chickenpox; one developed the disease thirty-six days after vaccination.

These investigations indicate that the virus of chickenpox is contained in the vesicle fluid; that direct cutaneous inoculation is possible and that active immunization may be effected. The disease is usually so mild, however, that vaccination may not be required as a practical measure.

VACCINATION AGAINST MEASLES

Immunity in Measles.—Measles occurs only in man; the lower animals commonly escape infection, but monkeys have been infected artificially by Anderson and Goldberger.⁵

The disease is most common between one and ten years of age. In rare instances children have been born with the disease, but infants under six months are rarely attacked and apparently enjoy for a period of six to ten months a congenital maternal immunity. Adults who have escaped the disease during childhood may contract it in later years.

Both sexes and all races are susceptible. Measles is probably the commonest of all the acute exanthemata. In communities first attacked by the disease, as the Faroe Islands and Iceland, measles has proved a virulent infection, suggesting a gradual immunization of the population where the disease is endemic.

One attack usually protects for life, but affords no protection against rubella (German measles).

Vaccination.—Children under five months of age are relatively immune to measles and a gradually diminishing degree of resistance persists for the first three or four years of childhood. Owing, however, to the mortality of this disease and particularly when complicated by pneumonia and other secondary infections, attempts have been made to develop a process of vaccination. Hermann⁶ has vaccinated 40 infants five months or less of age by inoculating the nose with virus obtained from the nasal secretions of children with measles twenty-four hours before the eruption and believes that protection was afforded. Vaccination in measles, however, is in the

¹ *Monatschr. f. Kinderh.*, 1914, 13, 205.

² *Archiv. Pediat.*, 1915, 32, 651.

³ *Boston Med. and Surg. Jour.*, 1917, 702.

⁴ *Amer. Jour. Dis. Child.*, 1918, 16, 34.

⁵ *Public Health Reports*, 1911, No. 26.

⁶ *Archiv. Pediat.*, 1915, 32, 503.

experimental stage. Better results have been reported following the injection of convalescent serum for prophylactic purposes (see Chapter XL).

VACCINATION AGAINST YELLOW FEVER

Immunity in Yellow Fever.—Up to the present time this disease has been observed only in human beings, the lower animals escaping even when intimately exposed during epidemics. Noguchi has recently claimed the successful inoculation of guinea-pigs by the injection of blood from individuals with the disease.

The negro is less susceptible than the white. Both sexes are equally susceptible. The disease is most common between ten and thirty years, young children and the aged frequently escaping. The virus is transmitted by the bites of infected mosquitoes (*Stegomyia jasciata*) and eradication of these mosquitoes and other hygienic measures are rapidly ridding the world of this pestilence.

One attack generally confers an immunity, but second attacks are not unknown.

Vaccination.—According to the French Commission, a certain degree of immunity could be conferred by the injection of serum heated to 55° C. for five minutes from yellow fever patients; some success has also been claimed for the injection of defibrinated blood kept under anaërobic conditions for eight days. They also claimed that the serum of convalescents had prophylactic and curative properties.

Noguchi and Pareja¹ have reported that guinea-pigs inoculated with sufficient amounts of killed culture of *Leptospira icteroides* are usually rendered resistant to a subsequent infection with living cultures. Encouraging results have been observed in the vaccination of human beings, but many more observations will be required before a final decision can be reached regarding the value of vaccination against yellow fever with leptospira vaccines.

VACCINATION AGAINST SYPHILIS

Immunity in Syphilis.—A great deal of attention has been devoted to studies in immunity in syphilis since the discovery of the *Spirochæta pallida* in 1905, and the successful transmission of the disease to apes and rabbits; the subject is one of importance owing to the wide-spread prevalence of the disease and especially in relation to diagnosis and treatment.

Natural immunity to syphilis does not appear to exist among human beings. All races are susceptible to infection, although it would appear that the severity of the disease is lessened among those peoples where syphilis is wide-spread and known to have been present for centuries. Of course not all exposed individuals contract the disease. The greatest single factor of resistance is an intact epithelial barrier of the skin and mucous membranes. It would appear that infection requires a break in this barrier before *Spirochæta pallida* may penetrate to the deeper tissues and produce infection, but the abrasion may be so small as to escape detection and, indeed, may not be necessary at all, in view of the recent experiments of Reasoner and of Brown and Pearce, who report the successful infection of the genital organs and eyes of normal stock rabbits by the simple procedure of applying virulent *Spirochæta pallida* to the mucous membranes.

Fortunately, *Spirochæta pallida* cannot long exist outside of the tissues and syphilis does not occur spontaneously among the lower animals. Apart from monkeys and rabbits, the lower animals resist infection, although

¹ Jour. Amer. Med. Assoc., 1921, 76, 34.

syphilitic keratitis is claimed to have been produced in cats, sheep, and dogs by inoculation with virulent spirochetes. My own attempts to produce syphilis in white rats, guinea-pigs, and dogs by testicular inoculations have always failed, even when attempts were made to break down the resistance of the tissues and especially of the lymphocytes by preliminary exposure of the tissues to α -rays.

The nature of natural immunity in the lower animals is not known. The blood may be and usually is lacking in spirocheticidal properties and demonstrable antibodies; the leukocytes and other cells do not appear to possess the power of phagocytosis or but to a very limited degree. The spirochetes simply die off as if lacking a suitable pabulum analogous to the death of transplantable mouse cancer cells in a rat or other animal and explained by Ehrlich with his athreptic theory of immunity.

Human beings have a defensive mechanism against syphilis sufficient for offering some resistance to *Spirochæta pallida* after infection has occurred, but these factors are not nearly so effective as in the monkey and rabbit and infinitely less than in other of the lower animals. As long as living spirochetes are present in the tissues these defensive forces may be and usually are apparently sufficient to protect against superinfection, and to some extent prevent the extension of the disease, but they are not sufficient for the killing of the spirochetes with the production of spontaneous cure and do not leave a state of immunity to reinfection when sterilization is brought about by the administration of mercury and arsphenamin. Brown and Pearce¹ have likewise found that a rabbit once infected continues to harbor virulent spirochetes for the balance of life, and the defensive forces against syphilis are apparently better developed in this animal than in human beings, in view of the usual mildness of the disease and long periods of latency.

During the primary incubation period of syphilis, that is, the time elapsing between exposure and infection and the development of the chancre, the human being, monkey, and rabbit may be reinfected; for a brief period after the development of the sore auto-infection is possible. But with the complete development of the chancre resistance to superinfection and auto-infection becomes operative and persists in the great majority of syphilistics until cure or complete destruction of spirochetes is brought about by drug treatment. Instances of superinfection during the secondary stage of syphilis are practically unknown; during the tertiary stages it has occurred, but is relatively rare. As Neisser² states, the only human beings possessing resistance to syphilis are those who are syphilitic and harbor spirochetes in their tissues. He quotes Rollet as follows: "Although I and my predecessors have a thousand times attempted to reinoculate luetic subjects, we have never observed a successful case. I know no single fact more thoroughly proved than the insusceptibility of a syphilitic to the action of a new virus, and, moreover, these experiments are so harmless that they may be performed without scruple." Finger and Landsteiner,³ however, believe that resistance during syphilis is less absolute than commonly believed and that especially during the tertiary stages superinfection is possible; according to Landsteiner⁴ the new lesions will not take the form of a chancre, but appear in the form of the lesions being presented by the patient at the time of reinfection. John⁵ also cites a series of cases carefully collected from the literature of apparent

¹ Jour. Amer. Med. Assoc., 1921, 77, 1619.

² Beiträge zur Pathologie und Therapie der Syphilis, Berlin, Julius Sprinzer, 1911; Arch. f. Dermat. u. Syph., 1906, 78, 335; *ibid.*, 1906, 81, No. 1.

³ Verhandl. d. deutsch. Dermat. Gesellsch., 1907, 25.

⁴ Centrallbl. f. Bakteriöl., Ref., 1908, xli, 785.

⁵ Samml. klin. Vortr., Volkmann, 1907-09, 559.

reinfection during syphilis, but it is possible and, indeed, probable that some of these were reinfections of cured rather than uncuredluetics. John mentions that the severity of the reinfections was similar to the first attack, suggesting that the first infection did not engender sufficient antibodies to mitigate the severity of the reinfection.

Very probably the escape of some individuals from syphilitic infection after intimate exposure with the evidence of confrontation, is due to the fact that they are congenital syphilitics. I have known of 2 such cases in young men seeking advice under these conditions, who escaped infection only to learn of being congenitally syphilitic and both of whom presented syphilitic keratitis.

The well-known resistance to syphilis of a child born of a syphilitic mother (Profeta's law) and of an apparently healthy mother exposed to an actively syphilitic child (Colles' law) are not now believed due to an actual immunity to the disease. The Wassermann reaction has revealed that the child in the first instance and the mother in the second are actually syphilitic in most instances and that their resistance is simply the resistance to reinfection caused by the presence of latent syphilis. It cannot be denied, however, that antibodies are produced in the course of syphilis, and these are probably largely responsible for the periods of latency with freedom of symptoms; it is possible that these antibodies generated in a syphilitic mother may be transferred by the placenta to the child so that the resistance of the latter is passive, but experimental evidence indicates that the degree of this immunity is probably feeble and temporary and that the only sure and lasting resistance to infection with *Spirochæta pallida* is pre-existing syphilis.

Immunity to syphilis apparently does not persist after complete cure as usually occurs in smallpox, measles, typhoid fever, etc. As previously stated there is no conclusive evidence to indicate that syphilis is self-curative or self-limited. It appears that antibodies and other factors of resistance may limit the development of lesions and maintain variable periods of freedom from symptoms, but actual and complete destruction of spirochetes probably does not occur without the administration of antiluetic drugs. In some races, and notably the negro, these defensive agencies may be particularly well developed, as, likewise, in women infected by giving birth to syphilitic children, but the latter is probably concerned more with phases of the cycle of infection than processes of immunity. Since invasion of the brain and cord with *Spirochæta pallida* occurs in probably the majority of cases of early syphilis, and the incidence of late symptomatic and asymptomatic neurosyphilis is limited, it is probable, as recently stated by Keidel,¹ that these immune reactions tend to protect the neuraxis.

It appears that human individuals are much less able to develop immunity to protozoan than to bacterial infections, and *Spirochæta pallida* is now classed by most biologists as a protozoön. In this regard the immunity developed in syphilis is similar in its feebleness to immunity in other protozoan infections, as trypanosomiasis and malaria. It would appear that in general terms the lower in the scale of life the infectious agent, the more complete our processes of immunity; immunity is usually more complete in bacterial than in mycotic infections and more complete in mycotic than in protozoan infections. For this reason the writer surmises that the infectious agents of smallpox and measles belong to a very low order by reason of the completeness of the immunity following these infections. Furthermore, the production of antibodies for *Spirochæta pallida* by the injection of vaccines of the spirochetes is only slight, and up to the present

¹ Jour. Amer. Med. Assoc., 1922, 79, 874.

time no efficient means of prophylactic immunization has been evolved; this subject will be reviewed in more detail later in the discussion on vaccine therapy in syphilis. Likewise all attempts to confer passive immunity by injections of blood from luetic subjects during the latent periods when antibodies should be expected to occur in the blood in largest amounts, have usually failed; this subject will also receive more discussion shortly.

Antibodies, however, do develop during syphilis of the human being and lower animals, but the amounts are slight and at best only capable of holding the disease in check and preventing reinfection without persisting after cure by drugs or bringing about spontaneous cure. Agglutinin production has already been discussed in Chapter XV; slight amounts of precipitin may also be produced as discussed in Chapter XVII. With cultures of *pallida* Broadwell and myself¹ were unable to discover appreciable amounts of spirocheticidal substances in the sera of syphilitics, but Eberson² has recently claimed that substances of this nature are to be found and especially in the latent and tertiary stages of human syphilis. Phagocytosis does not appear to be an important phenomenon in syphilis, although the spirochete has been found by Levaditi and others within body cells; very little work has been done on the question of immune opsonins for *pallida*, but in my own experiments with the sera of luetics these were not found to be increased.

Most attention has been given the Wassermann reaction in reference to antibody production in syphilis, but as discussed in Chapters XXIII and XXIV the substance in the blood and spinal fluid of the syphilitic responsible for the complement-fixation and flocculation reactions are not antibodies in the sense of being destructive for the spirochetes; they appear to be peculiar cellular products endowed with the property of bringing about flocculation of lipoids in colloidal states with the absorption or fixation of complement. Specific complement-fixing antibodies in syphilis for antigens of *Spirochæta pallida* are developed only to a slight degree and not at all to an extent comparable to the production of the cellular product responsible for the Wassermann reaction.

Resistance or immunity in syphilis presents other peculiar features. For example, the luetic after the primary stage is resistant to reinfection, but at the same time is subject to exacerbations of his own disease. In other words, new spirochetes are apparently unable to gain a foothold in his tissues, but his own spirochetes may progressively invade other organs and tissues. If different strains of *Spirochæta pallida* exist, as indicated by the work of Noguchi,³ Nichols,⁴ Levaditi and Marie⁵ and Marie, Levaditi, and Banu,⁶ infection with one strain appears to protect against other strains, although, if Landsteiner is correct, that the uncured syphilitic can be reinfected more frequently than surmised, it may be that just the reverse is true, namely, that the uncured syphilitic is resistant to spirochetes of his own strain, but susceptible to other strains.

The nature of this resistance of the syphilitic to reinfection and the resistance offered to the disease and responsible for the varying periods of latency is not well understood. Unquestionably a local tissue immunity develops and antibodies or other resistant substances are produced locally in a syphilitic lesion capable of protecting other organs. For example,

¹ Jour. Immunology, 1916, 1, 429.

² Arch. Dermat. and Syph., 1921, 4, 490.

³ Jour. Exper. Med., 1912, 15, 201.

⁴ Jour. Exper. Med., 1914, 19, 362; Jour. Amer. Med. Assoc., 1916, 67, 1799.

⁵ Ann. de l'Inst. Pasteur, 1919, 33, 741.

⁶ Compt. rend. Acad. d. Sci., 1920, 170, 1021.

Nichols¹ found that an active lesion in one testicle of a rabbit tends to inhibit the development of lesions in the other testicle or other parts of the body. Kraus and Volk² has previously demonstrated a local immunity of the skin, and Zinsser, Hopkins, and McBurney³ observed that while the second normal testicle of a rabbit may be infected during or after the infection of the other testicle, that the latter could not be reinfected and constantly exhibits a well-marked degree of local resistance.

Undoubtedly antibody production occurs in the tissues involved in the chancre responsible for the general resistance to reinfection and auto-infection exhibited in the primary stage of syphilis.

Brown and Pearce⁴ have recently shown in the rabbit that any procedure, as castration or the administration of antisyphilitic drugs, tending to reduce or suppress the development of the local lesion tends to intensify the general infection. These investigators have shown⁵ that measures of this kind modifying the local primary reaction between spirochetes and tissue cells may modify the clinical course of syphilis in the rabbit, and that this may be more important in relation to the different clinical types of syphilis in the human being than the question of different strains of *Spirochæta pallida*. Levaditi⁶ has shown many years ago that spirochetes may be found in the spleen of the monkey even before the primary lesion is well developed, and Brown and Pearce⁷ have shown that the blood and lymphatic systems of rabbits may be invaded before the initial lesion can be detected. These observations indicate that syphilis becomes generalized very early in the primary stage and that surgical removal of a chancre alone has little chance of preventing general infection. While the work of Brown and Pearce⁸ indicates that surgical removal of the primary lesion aids in the dissemination of syphilis of the rabbit probably by removing a factor for the production of immunity principles, and that the local struggle between parasites and host determines in an important manner the subsequent dissemination of the disease, there can be little doubt of the wisdom of destroying the spirochetes in human chancres as quickly as possible by the prompt administration of adequate amounts of antispirechetic drugs.

Antibody production in syphilis, therefore, appears to take place only in the tissues directly invaded by living spirochetes; there does not appear to be a general antibody production through stimulation of the antibody tissues in general by means of some diffusible toxin or other antigenic stimulant short of the living parasites themselves. The immune substances produced in foci of infection are, however, generally distributed and may offer an effectual resistance to reinfection, but the highest degree of resistance is found in the foci themselves where presumably the largest amounts of antibodies are to be found. Eberson and Engman,⁹ Eberson,¹⁰ Brown and Pearce,¹¹ and others have expressed the opinion that the latent foci may elaborate antibodies and that latency itself connates a balance between spirochetes and antispirechetic substances. Unfortunately, however, the latter substances are not usually able to kill the spirochetes and the latter

¹ Jour. Amer. Med. Assoc., 1914, 63, 466.

² Wien. klin. Wchn., 1906, 19, 621.

³ Jour. Exper. Med., 1916, 23, 329, 341.

⁴ Arch. f. Dermat. u. Syph., 1920, 2, 675.

⁵ Ibid., 1921, 3, 254.

⁶ Ztschr. f. Immunitätsf., Ref., 1910, 2, 277.

⁷ Arch. f. Dermat. u. Syph., 1920, 2, 470.

⁸ Arch. f. Dermat. u. Syph., 1921, 3, 254.

⁹ Jour. Amer. Med. Assoc., 1921, 76, 160.

¹⁰ Arch. f. Dermat. u. Syph., 1921, 3, 111.

¹¹ Arch. f. Dermat. u. Syph., 1920, 2, 470.

may spring into activity as a result of trauma or other cause, and as the years go by progressively involve new organs and new tissues of the same organ.

The question now arises regarding the nature and mechanism of the apparent resistance to reinfection shown by the syphilitic which develops during the primary stage and persists in a marked or absolute degree for the balance of life or until a complete cure is brought about by antisymphilitic remedies. When the uncured syphilitic is exposed to reinfection what seemingly prevents a new infection? Surely reinfection would seem possible when even in the old foci antisyphilitic substances are not sufficiently powerful or numerous to effect sterilization; probably it is a matter of numerical infection, that is, the syphilitic carries sufficient antisyphilitic agents to kill the relatively few spirochetes applied to the skin or mucous membrane upon exposure and thereby prevent reinfection, but these are insufficient to kill off all of the very large numbers of spirochetes of the previous infection now distributed in various foci throughout the body. If such were true it may be possible to break down this weak but usually sufficient resistance to reinfection by using spirochetes of enhanced virulence or in extra large numbers, and, indeed, animal experiments have indicated that this may be the case. Or it may be that there is no effectual resistance to reinfection at all in syphilis after the primary stage, meaning that new spirochetes may enter the tissues of the syphilitic upon exposure or inoculation, but simply mask their entrance by failure of the body cells to produce a local inflammatory reaction—a state of “anergie” according to Neisser. It may be that the local inflammatory reaction, the chancre, for example, is due to the irritant action of toxic substances elaborated by spirochetes and that in syphilis there is sufficient antibody to neutralize these and thereby prevent inflammation, although the spirochetes themselves are not killed and successively invade the tissues with subsequent distribution by lymphatic and vascular channels.

The whole subject of resistance and immunity in syphilis may be summarized as follows:

1. Natural humoral immunity to syphilis does not exist in human beings, although effectual resistance to infection may be offered by intact epithelial barriers.

2. After infection has occurred antibody production takes place in the foci of disease.

3. These antibodies may be sufficient for holding the spirochetes in a state of inactivity or latency, but are not usually sufficient for killing all of the spirochetes and, therefore, are unable to effect spontaneous cure unaided by medicinal treatment.

4. These antibodies do not persist except for a brief and unknown period after complete cure of syphilis; syphilis, therefore, does not leave any immunity or more than a brief and temporary immunity after complete destruction of all spirochetes.

5. A second chancre cannot usually be produced in an uncured syphilitic after the primary stage. Resistance to second chancres is practically absolute during the secondary stage, but may be produced in rare instances in the tertiary stage.

6. This resistance of the uncured syphilitic to a new chancre is due either to (a) the destruction upon exposure of the new spirochetes by the antibodies engendered by the previous infection, but which antibodies are unable to completely destroy the original spirochetes in the old foci of disease or, (b) the antibodies previously produced are able to neutralize the toxic products of the new spirochetes and thereby prevent a local tissue reaction

(the chancre or primary sore) without, however, actually destroying the spirochetes themselves and thereby permitting the latter to enter the tissues without local disturbances.

Vaccination Against Syphilis.—Owing to the prevalence and economic importance of syphilis it is not surprising that early and frequent attempts were made to evolve a method of prophylactic immunization. Up to the present time the results have been largely negative and an efficient method has not been discovered.

Metchnikoff and Roux¹ employed filtered virus and virus heated at 57° C. as vaccines for the immunization of monkeys, but with negative results in that treated animals were invariably infected as successfully as untreated controls. Neisser and Bruck² employed phenol-killed vaccines of the virus, with similar results. Uhlenhuth and Mulzer used without success living spirochetes from rabbit testicular lesions by intravenous and subcutaneous injection; Noguchi has employed vaccines of pure culture of pallida for the immunization of rabbits, observing that susceptibility to infection was reduced in some of these rabbits, but without influence in others, and with generally negative results. I have repeatedly tried immunization of rabbits with living and heat-killed vaccines of culture pallida and susceptibility to testicular inoculation remained practically undiminished.

Spitzer³ has injected individuals subcutaneously with emulsions of human chancre material after the primary chancre had developed, and noted in some individuals that generalized syphilis was prevented, but these results were not confirmed by Neisser with monkeys or by Brandweiner⁴ and Kreiblich⁵ in man.

Passage of *Spirochæta pallida* through monkeys and rabbits may lower its virulence for man and by producing a mild lesion lead to the development of immunity to syphilis. Metchnikoff and Roux have recorded an accidental laboratory infection with a monkey strain in which the chancre was mild and atypical and generalized symptoms did not develop. A similar result was observed by them in the inoculation of a volunteer seventy-nine years old with a strain of pallida carried for five generations in lower monkeys. But passage through monkeys and rabbits may not sufficiently reduce virulence for man, as reported by Graetz and Delbanco⁶ in an accidental laboratory infection.

At any rate it would appear that nothing less than living spirochetes are necessary for immunization; furthermore, that these must produce a local lesion and continue to live before immunity becomes apparent. In all probability these attenuated spirochetes may later acquire enhanced virulence. The immunity does not appear to be more than that developing in the course of syphilis, and since animal passage does not appear to safely modify or permanently attenuate spirochetes in a manner analogous to the passage of smallpox virus through cattle, and since vaccination with killed spirochetes has failed to immunize, it may be said that prophylactic immunization against syphilis is not possible by present methods.

VACCINATION AGAINST WEIL'S DISEASE (INFECTIOUS JAUNDICE)

The cause of this disease is *Spirochæta icterohæmorrhagica* discovered by Inada. The disease is particularly prevalent among the miners of Japan,

¹ Ann. de l'Inst. Pasteur, 1903, 17, 809; *ibid.*, 1904, 18, 1, 657; *ibid.*, 1905, 19, 673; *ibid.*, 1906, 20, 785.

² Beitr. z. Path. u. Therapie d. Syph., 1911, 203–216.

³ Wien. klin. Wchn., 1905, 45; *ibid.*, 1906, 38.

⁴ Wien. klin. Wchn., 1905, No. 45.

⁵ Wien. klin. Wchn., 1906, No. 8.

⁶ Med. Klin., 1914, 375, 420.

the rat being a carrier and source of infection. Inada and his co-workers have grown the microparasite artificially and were able to actively immunize the lower animals. In a recent review of the subject Inada¹ states that good results have been observed by Ito, Matsuzaki, and Wani in the prophylactic immunization of human beings. The vaccine is prepared in the usual manner to contain 50,000,000 to 75,000,000 of spirochetes per cubic centimeter, and is administered in two doses of 1 and 2 c.c. by subcutaneous injection.

PROPHYLACTIC IMMUNIZATION AMONG THE LOWER ANIMALS

Since discoveries in bacteriology and immunity have usually been intimately associated with animal experimentation, it is not strange that the lower animals should have been the first to benefit from the knowledge thus gained. As a consequence, vaccine therapy, both prophylactic and therapeutic, is being extensively used in veterinary practice with good results.

VACCINATION AGAINST ANTHRAX

This was one of the first vaccines studied by Pasteur, and as a prophylactic measure it has proved of great value. It finds its greatest field of usefulness in case of an outbreak of anthrax, when it is used to protect the uninfected members of a herd, as well as any animals pasturing on infected areas.

In preparing the vaccine Pasteur was hampered by the fact that the spores of anthrax bacilli retain the virulence of the original bacilli. As the result of extended experiments, however, he discovered a means of attenuating the virulence of cultures by growing the bacilli at a temperature of 42° C.; he also found that inoculation with these attenuated bacilli would effectively vaccinate sheep and cattle, and so protect them against an attack of the disease.

One of the most dramatic stories² in the history of science is the account of the method by which Pasteur demonstrated his discovery to the public. Certain harsh critics, having heard of Pasteur's ability to prevent anthrax in laboratory experiments, and anxious to humiliate him, sent him a public challenge to demonstrate the experiment on a practical scale at a farm in the country. A number of farmers offered to place 60 sheep at his disposal. The challenge was immediately accepted, and Pasteur mapped out a plan of action, in which he safe-guarded himself by making no half-statements, but boldly promised complete success. Of the total number, 25 sheep were to be vaccinated and 25 were to remain unvaccinated. A fortnight later all 50 were to receive a lethal dose of the fully virulent anthrax. He declared that the 25 non-vaccinated sheep would die, whereas the 25 vaccinated would remain alive and well. The remaining 10 sheep were to serve as controls.

The challenge and its acceptance were widely advertised in the journals, and Pasteur was made the subject for many witticisms. Excitement ran high, and a large crowd, comprised of physicians, veterinary surgeons, journalists, farmers, etc., accompanied Pasteur to the farm (Pouilly le Fort) where he was to make the final test by inoculating the deadly anthrax. One vaccinated animal developed a temperature overnight, a fact that caused Pasteur much anxiety. On going to the farm the next day, however, again followed by the crowd, he found all the vaccinated animals well! Of the unvaccinated, 22 were dead, and the others died during the following

¹ Japan Med. World, 1922, 2, 189.

² Narrated by Elizabeth Fraser.

night. Pasteur's triumph was complete, and the possibility of preventive vaccination was demonstrated to the world.

Preparation of Vaccines.—The vaccine is prepared of attenuated cultures of virulent anthrax bacilli according to the method of Pasteur:

Vaccine No. 1 is weakest or lowest in virulence, and the first to be injected. This vaccine is prepared by growing virulent anthrax bacilli at a temperature of 42° C. for from six to ten weeks, or until 1/10 loopful of the culture, when injected into rabbits, guinea-pigs, and mice, will show virulence for mice only, but not for guinea-pigs and rabbits.

Vaccine No. 2 is prepared by growing virulent anthrax bacilli at 42° C. for about twenty days, or until 1/10 loopful is virulent for mice, partly so for guinea-pigs, and not at all for rabbits.

Vaccine No. 3 is not generally used except for immunizing sheep and goats. When, however, it is required, it is made as follows: Virulent anthrax bacilli are grown at 42° C. until 1/10 loopful, when injected into mice, guinea-pigs, and rabbits, will be virulent for all the mice, all the guinea-pigs, and some of the rabbits.

The vaccines are prepared in ampules containing 1 c.c. of the emulsion, each representing one dose, to be injected subcutaneously. Vaccine No. 2 is injected ten to twelve days after Vaccine No. 1, and No. 3 after the same interval following Vaccine No. 2. The resulting immunity usually lasts about six to twelve months.

At the present time anthrax vaccine is generally prepared by cultivating the bacilli and spores on agar for about five days at 38° C. followed by suspending them in saline solution and heating at 60° C. for one hour. A count is then made of this stock suspension by plating methods and dilutions prepared preserved with tricresol to give vaccines of approximately these strengths:

No. 1, No. 2, and single vaccine to contain from 1,000,000,000 to 2,000,000,000 per cubic centimeter.

No. 3 and No. 4 to contain from 2,000,000 to 5,000,000 per cubic centimeter. No. 4 is only occasionally used in localities where the incidence of anthrax is high and the maximum degree of immunization is demanded.

The vaccines are also available in pellet form which are placed in the subcutaneous tissues by means of a special injector.

Technic of Vaccination.—The animals about to be vaccinated should be tagged, numbered, described, and the temperature of each should be taken for additional assurance that they are not affected with anthrax. If the stable or other premises are in an unsanitary condition the animals should be removed to a clean, open lot for vaccination, in order to avoid risk of wound infection or contamination of the vaccine. This precaution is imperative.

It should be carefully remembered that *the vaccine is living* and the utmost care should be taken to avoid the loss of a single drop.

The site of injection, usually behind the shoulders (first injection upon the right and the second upon the left side), should be cleansed by scrubbing with soap and water and, after drying with swab of clean cotton or cloth, the area through which the needle is plunged should be disinfected with tincture of iodine or other reliable germicide. The dose of vaccine, consisting of 1 c.c., should be injected under the skin and the needle, on withdrawal, should be wiped and placed in a pledget of cotton moistened with tincture of iodine until the next injection is about to be made. Otherwise a sterile needle should be used for each animal. The used needle should be taken from the syringe with a pair of sterile forceps and a sterile needle

replaced in the same manner. Horses and mules should be injected under the skin of the neck, above the collar space. Draft animals usually need not be withdrawn from their work during vaccination and cattle in dairy herds may be vaccinated without affecting the milk supply.

Animals should not be immunized within two weeks of an operation. During hot weather the injections should be given, as far as possible, during the morning or evening hours. As a general rule pregnant animals may be immunized as there is little danger of abortion if rough handling is avoided. Animals that show symptoms of the disease should not be vaccinated.

Dosage.—Under ordinary conditions one dose of vaccine (No. 1) will protect animals for about one year. Preference should always be given, however, to double vaccination, the second dose (No. 2) following seven to ten days after the first. This immunity is more complete and durable.

In badly infected districts a third dose (No. 3) should be given about ten days after the second.

Vaccinated animals should not be turned into infected pastures for at least two weeks after the last injection, as it takes about this period for immunity to develop.

In instances where it is desirable to immunize a herd before turning them out to pasture on infected areas it is well to inoculate the animals in the early spring, keeping them in the stable during the time required for at least two vaccinations, for the reason that, immediately after vaccination, the animals may become hypersusceptible to infection.

Vaccination with Vaccine and Serum.—When *immediate* protection is required it is advisable to inject at least 10 c.c. of antianthrax serum subcutaneously followed at once by the subcutaneous injection of 1 c.c. of vaccine (No. 3) at another site. The two should not be mixed, but injected separately.

Practical Value.—When conducted in a careful manner by a competent veterinarian anthrax vaccination has proved fairly successful.

The immunity usually lasts for eight to twelve months at least.

Vaccination is not without danger. Some animals may die as a result of the injections. These are usually hypersusceptible to anthrax and are beyond control. Severe reactions of this kind may be controlled by the prompt intravenous injection of antianthrax serum in curative doses (30 to 100 c.c.).

VACCINATION AGAINST BLACKLEG (SYMPTOMATIC ANTHRAX)

Blackleg vaccine is used entirely as a prophylactic agent, for the disease runs too acute a course for the vaccine to exert any therapeutic influence.

The vaccine as employed at the present time is in the nature of an aggrassin previously described.

Preparation of Aggrassin.—Young cattle over six months of age are injected into the gluteal muscles with approximately 40 gm. of dried muscle virus. As a general rule the animals succumb in thirty-six to forty-eight hours. Immediately after death the infected tissues are removed and the bloody serous fluid collected. The muscle is then ground and the juice expressed and collected. This is added to the serous fluid and the whole preserved with 0.3 per cent. tricresol, filtered through candles and tested for sterility by aerobic and anaerobic cultures. Guinea-pigs are also injected with 5 c.c. amounts and should survive. The finished product has a clear cherry red color and after standing may show a slight sediment.

The vaccine is prepared by the Bureau of Animal Industry in the fol-

lowing manner: The muscle tissue from a fresh blackleg tumor is ground in a mortar, extracted or macerated with a little water, and the fluid squeezed through cheese-cloth. The expressed fluid is then evaporated at a temperature of 35° C. The dry brown scale is run through a grinding mill and heated for six or seven hours at a temperature of from 94° to 96° C. This process of heating attenuates the virulence of the bacilli present, so that when injected they produce but a mild attack of the disease. The Department of Agriculture places the ground material in packages containing a certain number of doses. These packages are, upon request, mailed to veterinarians, who dilute the ground muscle with as many cubic centimeters of sterile water as there are doses in the package. One cubic centimeter of the suspension is injected subcutaneously in some convenient area, as, *e. g.*, about the shoulders.

Blackleg vaccine should be applied in the spring, before young cattle are turned out to pasture on infected areas; the injections are subcutaneous. Reactions seldom occur.

In case of a fresh outbreak, all the healthy animals in the herd are to be vaccinated as soon as possible; sick animals are never to be injected.

Blackleg vaccination has been fairly successful, and usually confers an immunity lasting for a period of about six to nine months.

In young animals (up to two years) the immunity is of shorter duration than in older animals. After two years a natural immunity gradually develops.

VACCINATION AGAINST INFECTIOUS ABORTION OF COWS

The primary and essential cause of infectious abortion of cows is now regarded as the *Bacillus abortus* of Bang which causes an infection of the placental tissues and fetal membranes (chorion). Infection probably occurs through the intestinal tract followed by bacteremia and may take place at any stage of pregnancy. The bull seldom transmits the disease, but possibly may do so in a mechanical manner or as a result of infection of the testicles. Pus producing bacteria and certain fungi may also produce abortion, but the bacillus of Bang is the most frequent cause and the diagnosis is facilitated by the complement-fixation and agglutination tests previously described. Calves born of infected cows may harbor the bacilli for a short time, but immunity is not inherited and natural immunity to the disease is uncommon. Acquired immunity gained by the cow passing through an attack usually lasts for the balance of the animal's life.

Dosage and Administration.—Two vaccines are being employed for the immunization of cattle. A vaccine of killed bacilli may be injected subcutaneously in a single dose of 20 c.c. or better, in three or four doses of 5 c.c. each at intervals of five to seven days. Each cubic centimeter usually contains about 25,000,000,000 bacilli. The results have not been as good as observed after vaccination with living vaccine, but killed vaccine may be employed in herds where actual abortions are occurring and a number of pregnant animals have been exposed to infection. It may be given early in pregnancy, or at time of herding, in 5 c.c. doses monthly until delivery.

Living sensitized vaccine affords better protection, but obviously is more dangerous. The dose is 20 c.c. by *subcutaneous* injection. *Only non-pregnant animals should be injected, and they should not be bred for at least two months*, to allow for the destruction of any living bacilli which may have reached the uterus.

The immunity following vaccination with killed or living vaccines usually

lasts for about one year, and in infected herds it is recommended that the vaccine be given after each calf is born.

Practical Value.—Vaccination with *killed vaccine* probably engenders some degree of immunity of short duration. Its use is advisable when the disease breaks out in a herd in order to give assistance to the pregnant animals in coping with the infection.

The *living vaccines* given at the right time (about two months before pregnancy occurs) affords a greater degree of protection. Huddleson has shown that vaccination of non-pregnant cows and heifers with these engenders an immunity sufficient to enable the animals to withstand intense exposures and the intravenous injection of living bacilli. A number of fairly well-controlled experiments have shown that in infected herds with 20 to 30 per cent. of animals aborting, immunization with living cultures reduces the incidence to about 5 per cent. Calving efficiency does not seem to be impaired.

When the disease breaks out in a herd the infected animals and all having vaginal discharge should be isolated. All fetuses and contaminated bedding, etc., should be burned and the stable thoroughly cleaned and disinfested. *Living vaccine* may be given all animals that have aborted or that freshen, as soon as they clean; also to all virgin heifers and newly purchased non-pregnant cows. These vaccinated animals should be bred for about two months. Heated or *killed vaccine* may be given all pregnant animals in the herd and all empty animals that must be bred at once.

VACCINATION AGAINST INFECTIOUS ABORTION OF MARES

This disease is now regarded as being caused by *Bacillus abortus equi*, first described by Smith and Kilbourne,¹ and quite different from the bacillus of Bang, responsible for the corresponding disease of cattle previously discussed. This bacillus is also regarded by many as the cause of infectious arthritis (joint-ill) of colts.

Good and Smith² have found that vaccination of mares before infection with killed vaccines engenders some degree of immunity and does no harm. Four subcutaneous injections of 5 c.c. each at intervals of five to seven days may be given. Immunization may also be effected by two injections of 10 c.c. each at intervals of five days or one injection of 20 c.c.

Schofield³ and Hardenbergh⁴ have observed good results in the prevention of arthritis of colts by giving several subcutaneous injections of vaccine in dose of 2 or 2.5 c.c. at intervals of two to five days.

VACCINATION AGAINST CANINE DISTEMPER

Canine distemper is now generally believed to be caused by *Bacillus bronchisepticus* discovered by Ferry.⁵ Other micro-organisms, as staphylococci and streptococci, are commonly found in the discharges, but are regarded as secondary invaders.

Dogs from one to twelve months of age are particularly susceptible; animals over three years old are rarely infected. The incubation period varies from two to eighteen days, and young animals that have come in contact with the disease within three weeks should be considered as possibly infected.

¹ U. S. Dept. Agriculture, Bureau of Animal Industry, 1893, Bull. No. 3, 49.

² Jour. Infect. Dis., 1914, 15, 347.

³ Toronto, Canada Govt., 1915.

⁴ Circular 48, Dept. Agriculture, Pennsylvania.

⁵ Amer. Vet. Rev., 1910, 499.

Vaccination with killed vaccines is regarded by some as affording protection for eight to twelve months and is probably worth while. For a dog of ordinary size three doses of 2 c.c. each (approximately 2,000,000,000 bacilli) may be given by subcutaneous injection at intervals of five to seven days. Puppies of about four to six weeks should receive smaller doses (0.5 to 1 c.c.).

VACCINATION AGAINST CANINE RABIES

As stated on p. 791, Semple, Umeno and Doi, Eichhorn and Lyon, have shown that one large dose of phenolized fixed virus by subcutaneous injection appears to protect dogs against street virus. The duration of the immunity is not definitely known. When dogs are bitten by rabid animals it may be worth while to give them this injection not only for the protection of the animal against the disease, but more particularly to protect human beings. Until the method is put on a more conclusive basis, however, it is advisable to quarantine bitten dogs for a sufficient time even though they are apparently immunized. Of course this is not necessary with dogs not bitten, but immunized for protection. However, if an injected dog is subsequently bitten by a rabid animal, he should be destroyed or very carefully quarantined.

OTHER DISEASES

Vaccination Against Hemorrhagic Septicemia or Pasteurellosis of Animals.—Since the discovery by Pasteur of the *Bacillus avisepticus* of fowl cholera, a similar bacillus has been isolated by many investigators in a similar disease of cattle, horses, buffalo, swine, sheep, goats, and rabbits. These bacilli are now regarded by some investigators as being the same micro-organism which, adapted to one species, can produce a disease peculiar to that species. The general symptoms and lesions of septic infections, as fever, difficult respiration, tremors, and edema of the region of the throat are much the same for all species.

Killed vaccines are being employed for vaccination against the disease occurring in cattle (*B. bovissepticus*), swine (*B. suissepticus*) and sheep (*B. ovissepticus*). For cattle 5 c.c. may be injected subcutaneously every five days for four doses or two injections of 10 c.c. each may be given at intervals of ten days, or one injection of 20 c.c. For swine and sheep four injections of 2 c.c. each may be given every five days or two injections of 4 c.c. each at ten-day intervals, or one injection of 8 c.c. Hardenbergh and Boerner¹ have advocated the use of living vaccines.

The duration of the immunity is short and infected herds require immunization every year; available reports indicate that the incidence of the disease is materially reduced by vaccination.

Vaccination Against Equine Influenza (Strangles of Horses and Mules; Influenza; Pneumonia).—Active immunization with killed mixed vaccines of *Streptococcus equi* of Schütz (etiologic agent of strangles), *Bacillus equisepticus*, and other micro-organisms found in influenza and pneumonia of horses and mules, is claimed by some investigators to be of prophylactic value. These vaccines are given subcutaneously in four doses of 5 c.c. each at intervals of five to seven days.

¹ Jour. Amer. Vet. Med. Assoc., 1917. xlix, 55.

CHAPTER XXXVI

PRINCIPLES OF PASSIVE IMMUNIZATION—SERA IN THE PROPHYLAXIS AND TREATMENT OF DISEASE

SERUM therapy may be said to have had its origin in 1890, when von Behring discovered diphtheria antitoxin. He found that guinea-pigs surviving a subcutaneous inoculation of living diphtheria bacilli may harbor virulent bacilli at the site of injection without showing any evidences of intoxication. Subsequent investigation showed that the blood-serum of these animals contained the protective principles, for when the serum was injected into other animals along with the diphtheria toxin symptoms of the disease did not develop, and, indeed, as was shown later, the immune serum was found capable of neutralizing the toxin in the test-tube. Shortly afterward Kitasato made similar discoveries in studying tetanus, and these antitoxins have since proved of great importance, not only from the new light that has been thrown upon the mechanism of immunity—and they were used as important arguments for the humeral as opposed to the phagocytic theory, and form the very basis and starting-point of Ehrlich's researches—but also from the new and important field of therapy that was now opened, which gave promise and hope for the discovery of a specific serum treatment for each bacterial disease.

At the time it was thought possible to immunize animals with the various micro-organisms known to produce disease, and that the immune serums so produced may be employed in the form of specific treatment. This theory rested on the fact that they contained the antibodies that would quickly overcome the infection. With a few of the genuinely antitoxic serums these hopes have been realized; but many other serums have not yielded the expected and wished-for results, although at the present time the reasons for failure are being recognized and gradually eliminated.

Definitions.—It will be remembered that in active immunization our own body cells are stimulated to produce antibodies, either by reason of the presence of a disease or as the result of vaccination with the antigen of the disease in a modified and attenuated form. *In passive immunization, however, our own body cells do not produce the antibodies, but we receive them passively in the form of an injection of an antibody-laden serum. The antibodies are produced by active immunization of some other animal, usually a horse, and we receive the antibodies or products of this immunization in a passive manner, i. e., our body cells receive protection against an infection and aid us in overcoming it through antibodies produced in some other animal.* For this reason the process is called *passive immunization*; the particular kind of increased resistance afforded against infection is known as *passive immunity*, and since blood-serum contains the antibodies and is the usual vehicle by which they are transferred, the method is called *serum therapy*.

Passive Immunization for the Prevention of Disease.—In *prophylactic immunization* the immune serum is introduced into our body fluids before infection has actually occurred, or at least in the earliest stage of infection, for the purpose of placing the antibodies on guard to destroy the infecting micro-organism or to neutralize its products before it has had an opportunity to produce disease. In other words, we aim to fortify our natural defenses by purchasing antibodies from another animal. From the fact that these

antibodies may be introduced in a short space of time and that in this manner an immunity may be quickly gained, passive immunization for prophylactic purposes is indicated when the danger of infection is imminent, and when it is impossible, or when there is not sufficient time, for us to stimulate our own body cells to produce our own antibodies by active immunization with a vaccine.

Since the antibodies are produced in another animal, the serum, when introduced into our body fluids, represents a foreign protein, and, accordingly, we find that the antibodies are retained for relatively short periods of time and are quickly eliminated or destroyed. In active immunization, however, the antibodies are in native surroundings, and our body cells continue to produce them for some time after active stimulation has ceased, in this manner insuring a higher degree of immunity and one of longer duration. *For purposes of prophylaxis, therefore, active immunization is always more desirable than passive immunization;* not infrequently the two forms are used simultaneously, as the antibody-laden serum will afford instant protection, while the vaccine is stimulating our body cells to produce antibodies that will increase and maintain the protection over a longer period of time. This mixed form of immunization has recently received special study by von Behring in immunization experiments against diphtheria, and will be considered in detail in a later section.

For *purposes of prophylaxis* only two immune serums have proved their efficiency, namely, the antitoxin of diphtheria and tetanus antitoxin.

As will be pointed out further on, diphtheria antitoxin, when administered in sufficient amounts, affords protection for at least from four to six weeks; mixed immunization, by means of the simultaneous injection of a neutral mixture of the toxin and antitoxin, has been found to yield equally good and more prolonged immunity.

Tetanus antitoxin has its greatest value as a prophylactic. When symptoms of tetanus have once appeared, serum treatment may be of no avail, whereas it has proved its efficiency beyond doubt in neutralizing the toxin before it reaches or unites with the nervous tissue. In all wounds likely to be infected with tetanus the physician should include the administration of tetanus antitoxin as a matter of routine treatment.

Of the antibacterial serums, many have a prophylactic value in experimental animals, but none, with the exception of the antiplague serum, is in general use as a prophylactic in human practice. The reasons for this are apparent when it is remembered that pneumococcus, streptococcus, and meningococcus infections are not sufficiently epidemic in character to demand passive immunization. Meningococcus meningitis may, however, be an exception, but the method of active immunization advocated by Sophian is promising, easier to carry out, and should be tried during times of epidemic meningitis.

In typhoid fever, cholera, and dysentery antibacterial serums have not been generally used in prophylaxis, although it would appear that a potent anticholera serum would prove of value in preventing epidemics of this frightfully infectious disease.

With the exception, therefore, of the true intoxications, prophylaxis is more readily secured by active than by passive immunization. This is certainly true of typhoid fever, rabies, and smallpox. The antisera of other micro-organisms, such as the pneumococcus, streptococcus, and gonococcus, are being used exclusively for therapeutics, rather than for prophylaxis, of their several infections.

In veterinary practice hog-cholera serum has proved of value as a prophylactic.

lactic means of combating and limiting epidemics of hog cholera. It is not definitely known whether this serum is antitoxic or antibacterial, but it is probably a combination of both.

Passive Immunization for the Treatment of Disease (Serum Therapy).—In *curative immunization* the conditions are somewhat different. During the course of an infectious disease our body cells are actively engaged in combating the infectious agent, so that reinforcements, in the form of specific antibodies, are indicated and welcomed for the aid they give in overcoming an infection and the relief they afford our hard-pressed protective mechanism.

For these reasons it may be stated that the more acute the infection, the greater is the indication for introducing an antibody-laden serum. In chronic infections and in some acute infections we may practice active immunization by introducing a vaccine, with the purpose in mind of stimulating dormant cells to produce antibodies; but, as a rule, it is reasonable to assume that in a severe generalized infection our body cells are doing their utmost to overcome the infection, and extra stimulation may be actually harmful. By introducing antibodies produced in some other animal, however, practically no extra strain is thrown upon the body cells; on the contrary, they may be relieved when the new antibodies overcome the products of infection, and in this manner afford them an opportunity to recover.

In the treatment of disease immune serums have proved of value in diphtheria, tetanus, cerebrospinal meningitis, and, to a lesser extent, dysentery, pneumonia, streptococcus infections, and plague.

While antipneumococcus, antistreptococcus, and antigenococcus serums have proved of some value in the treatment of their particular infections, the more recent work of Neufeld and Händel, Dochez and Cole, and their co-workers in pneumonia indicates that there are wide biologic differences among various strains of these micro-organisms, and that no curative properties can be expected from a given serum unless this is homologous for the type causing the infection. Further than this, it has been found impossible to secure serums as rich in antibodies as are secured with diphtheria and tetanus antitoxins, and that the serums must be given intravenously in relatively large doses. A method for the quick recognition of types of pneumococci has been worked out in the Rockefeller Hospital, and immune serums have been prepared for the main types, and the results of the serum treatment of pneumonia along these lines have been found to be most encouraging. While this method is not adapted for general use, it holds out a promise for the future of serum therapy, and opens up a wide field of investigation with the group of streptococci, gonococci, and meningococci.

Varieties of Passive Immunization.—While, strictly speaking, all antibodies are probably inimical to their antigens, from the practical standpoint of passive immunization three are of primary importance, namely: (1) The antitoxins, (2) the bactericidans and bacteriolysins, and (3) the bacteriotropins (immune opsonins). The antitoxins neutralize their toxins; bacteriolysins cause the death of their respective bacteria if suitable complements are present, and bacteriotropins accomplish the same end by lowering the resistance of the bacteria, and in this manner facilitating phagocytosis. Other antibodies may be operative and prove of assistance, as, *e. g.*, agglutinins may aid in bacteriolysis and anti-aggressins may aid in phagocytosis, but too little is known at the present time to allow fine distinctions to be made, although the indications are that *not one but several antibodies are present in each immune serum, which, acting together, tend to overcome an infection.*

From the practical standpoint, therefore, immune serums may be used to produce two main types of passive immunization, namely:

1. *Antitoxic immunization*, due to antitoxins for the true or extracellular toxins, as in diphtheria and tetanus (antitoxic immunity).

2. *Antibacterial immunization*, due mainly to bacteriolysins and bacteriotropins, as in meningococcus, pneumococcus, streptococcus, gonococcus, and similar infections (antibacterial immunity).

Kinds of Sera Employed in Serum Therapy.—(a) *Immune horse and human sera* are used for prophylactic immunization because of the large amounts of antibodies contained in them. These immune sera are commonly prepared by active immunization of horses, as in the preparation of the antitoxins, pneumococcus, and meningococcus antisera; goats and other animals are sometimes employed, notably oxen, in some European laboratories.

In addition to these immune sera of the lower animals, immune human sera are sometimes employed and especially in the treatment of scarlet fever and acute anterior poliomyelitis with the sera of persons convalescent from scarlet fever and recovered from poliomyelitis. These sera contain curative principles, but cannot be prepared artificially until the etiologic agents are available for the immunization of the lower animals. These human immune sera have been employed for prophylactic as well as therapeutic immunization; the injection of serum from convalescent cases of measles and mumps have been advocated for passive immunization against these diseases. The success of this serum prophylaxis in measles strongly suggests that a similar procedure may prove efficient for conferring passive immunity against scarlet fever.

(b) *Normal sera* are likewise employed in the treatment of some diseases. For example, the serum of cattle has been advocated for the treatment of human anthrax in the Argentine and probably owes part of its efficacy to the presence of natural antibodies inasmuch as the disease is very prevalent in that country and may result in the immunization of many of these animals.

Normal human serum has also been advocated for the treatment of hemorrhage and various skin and other diseases, but probably owes its efficacy largely to non-specific factors. Under this heading is likewise to be included the transfusion of blood for the treatment of hemorrhage and sometimes for severe pyogenic infections.

Mention is also to be made of the use of normal serum immunized *in vitro* by the method of Wright, and to which further reference will later be made.

(c) *Exudates and transudates* are likewise sometimes employed for the treatment of disease. The former may contain antibodies and notably bacteriotropins, but both probably owe their curative activities to non-specific factors, a form of non-specific protein therapy.

Specific and Non-specific Serum Therapy.—For prophylactic purposes, as in the prevention of diphtheria and tetanus, specific immune sera must be employed containing antibodies capable of neutralizing the bacterial poisons and aiding in the actual destruction of the microparasites. For the treatment of disease immune sera containing large amounts of various antibodies should likewise be employed for these purposes, but in addition to these specific activities, immune sera probably owe part of their efficacy to non-specific properties largely in the way of stimulating leukocytosis and an increase of natural antibodies and proteolytic ferments. Normal serum may owe part of its curative effects to the presence of natural antibodies,

but these are not sufficient for prophylactic immunization, although they may aid in the treatment of disease; normal sera probably owe their efficacy entirely to non-specific activities as previously mentioned and as will be discussed in more detail in Chapter XXXIX.

Duration of Passive Immunity.—Immune horse-serum is employed in human medicine for prophylaxis against diphtheria, tetanus, and gas gangrene; in veterinary practice against anthrax, symptomatic anthrax (black-leg), hog cholera, and a few other diseases. Convalescent human serum has been employed for prophylactic immunization against measles and mumps with encouraging success, and a similar prophylaxis may be developed for scarlet fever and other of the infectious diseases.

The duration of the immunity conferred by horse and human sera is quite brief and probably not more than six weeks even when homologous serum is employed. Passive immunization, therefore, has its greatest value for tiding over a period of emergency as in epidemics; for a more lasting immunity active immunization, by which our own body cells are stimulated and probably continue to produce protective principles, after the inoculations cease, is required, and is generally more effective.

Reactions After the Administration of Sera.—(a) The administration of *homologous sera as normal or immune human serum to a human being by subcutaneous or intramuscular injection* does not ordinarily produce more than a local reaction due to trauma and the irritation excited by the preservative; this is sometimes accompanied by mild fever that subsides within thirty-six hours. The local reaction is one of pain and tenderness accompanied by slight erythema and edema and the severity depends largely upon the amount of serum injected.

(b) *Homologous serum injected intravenously*, however, may excite in addition to this local reaction, a mild general reaction of chilliness, slight fever, and possibly tachycardia, if large amounts are administered. These effects may be due to intravascular agglutination and hemolysis unless the serum employed has been found free of agglutinins and hemolysins for the corpuscles of the patient. They may likewise be excited in part by sodium citrate or other anticoagulant, if these have been employed in the preparation of the serum. Finally this reaction may be caused by increased toxicity due to colloidal changes occurring during the preparation of the serum and the reaction closely resembles a mild protein shock; these factors are discussed in more detail in the chapter on Blood Transfusion.

The administration of homologous serum, however, does not produce serum sickness or other evidences of an anaphylactic reaction.

(c) *The subcutaneous and intramuscular injections of normal and immune horse-sera* do not ordinarily produce more than a local traumatic reaction, the severity depending largely upon the amount injected. Ordinarily this is accompanied by chilliness, mild fever, headache, and other general symptoms subsiding in thirty-six to forty-eight hours and a form of protein shock reaction.

Serum sickness may develop at a subsequent time and in rare instances a severe reaction of dyspnea, tachycardia, coma, and even death may be induced in those human beings who are "horse asthmatics" and acutely hypersensitive or anaphylactic to horse-serum protein (see Chapter XXX).

(d) *Intravenous and intraspinal injections of horse-serum* are usually followed by a reaction ascribed to the effects of the various foreign proteins. Sometimes and, indeed, not infrequently there is practically no reaction, and especially if it happens to be the first dose of horse-serum ever taken by the patient.

As a general rule, however, and especially if 50 c.c. or more of serum is administered intravenously, a reaction sets in from fifteen minutes to one hour later characterized by chilliness, slight dyspnea, and cyanosis. The temperature rises rapidly 1° to 3° F. and then falls, often to normal. Profuse perspiration may now take place. As shown by Matsumoto and the writer¹ the amounts of agglutinins and hemolysins for human erythrocytes present in horse-sera are probably too slight to induce reactions by intravascular agglutination and hemolysis, and especially if the serum is diluted and slowly injected.

Severer reactions may occur if the patient is hypersensitive to horse-serum proteins and serum disease may develop at a subsequent time. These reactions are described more fully in Chapter XXX, and their nature, means for prevention, and treatment should be thoroughly understood by every physician employing serum therapy.

Dangers and Contraindications to Serum Therapy.—*The chief contraindications to the therapeutic use of a serum are those dependent upon the serum itself*, for, as will readily be understood, the introduction of antibodies themselves does not mean an extra strain upon our body cells, but rather the reverse. The question before us, then, is one regarding the possible contraindications to the injection of a foreign serum, and the dangers dependent upon its use. It may be stated at once that, in the great majority of cases, *the administration of a carefully prepared and properly administered serum is free from danger*. Since the introduction of diphtheria antitoxin in the prophylaxis and treatment of that disease many thousands of injections have been given, in all parts of the world and under all sorts of conditions, and the number of fatalities is so small as to be regarded as almost negligible. Serum therapy should not, however, be abused to the extent of using the serum indiscriminately. I am opposed to using the serum as a prophylactic unless the indications for its employment are distinct; for example, in diphtheria it suffices to immunize only those who have been brought into immediate contact with the infection. When, however, the indications are clear and the symptoms of infection are present, I believe in using the serum early and generously.

1. Of all possible dangers consequent to the use of serum therapy, that of *anaphylaxis* is uppermost in the minds of practitioners. While it is true that anaphylaxis has been the cause of some fatalities, the likelihood of this accident taking place is so remote, in the great majority of cases, that it should not occupy a prominent place in the physician's mind, nor interfere with the use of the serum, as, for instance, antitoxin in the treatment of diphtheria. It is true that *serum sickness* is comparatively common, and while the symptoms are frequently distressing, they are not dangerous and do not constitute the dreaded and fatal anaphylaxis. With a little discrimination and care on the part of the physician the risk of anaphylaxis may be rendered still more remote if attention is given to the following questions:

(a) Is the patient sensitive to horse protein? This is probably the most important single question, as in several of the fatal cases of anaphylaxis on record it was learned afterward that the patient was usually rendered uncomfortable, and that sneezing, asthma, or even an urticarial rash would develop when the patient came into close proximity to horses, as in a stable, or when driving behind them, etc. Fortunately, these cases are very few, but several of the fatal cases of anaphylaxis on record occurred in just such persons, and at the present time a physician should generally be able to

¹ Jour. Immunology, 1920, 5, 75.

detect this susceptibility and avoid the dangers of anaphylaxis. In Chapter XXX the subject is discussed in greater detail, and skin tests are described in Chapter XXXI by which it may be possible to detect this condition.

(b) Has the patient been injected with a serum on any former occasion? If an injection has been given, especially a few weeks earlier, a reinjection of serum may cause well-marked serum sickness, but the possibilities of alarming anaphylaxis are so remote that serum should never be withheld if the clinical condition indicates that it should be given. Not infrequently a child receives an immunizing dose of diphtheria antitoxin, but develops the disease a month or two later, after the immunity has disappeared. Under these circumstances antitoxin should not be withheld. If time permits, the physician may inject 0.5 c.c. of the antitoxin for the purpose of producing *anti-anaphylaxis*, followed in two or three hours by the remainder of the serum. (See Chapter XXXII.) *If it were possible to obtain it, it would be good practice to immunize the patient with an ox-serum antitoxin, and then, if it was found necessary later to use an antitoxin, the usual horse-serum antitoxin could be employed.* This would still further eliminate the possibility of the development of disagreeable or dangerous complications.

2. If a patient suffers from idiopathic asthma and the condition known as status lymphaticus develops, serum should be given cautiously because of the increased respiratory difficulties that may follow. It may be well to give a preliminary hypodermic injection of atropin and caffein, and then, after a few minutes, give the serum, injected slowly and subcutaneously.

3. Aside from these questions, the physician may be called upon to decide if a patient is physically able to withstand the effects of an inoculation, especially the intravenous injection of relatively large amounts of serum, such as are given in the treatment of pneumonia. In diphtheria in very young and weak children, when a large number of units or several injections are to be given, concentrated antitoxin is to be preferred, in order that injury to the subcutaneous tissues, pain, and shock may be reduced to a minimum.

CHAPTER XXXVII

METHODS FOR THE ADMINISTRATION OF SERUM IN THE PROPHYLAXIS AND TREATMENT OF DISEASE

UPON the nature and severity of the infection will depend the question whether the serums are to be given *subcutaneously*, *intramuscularly*, *intravenously*, or *intraspinously* (*subdurally*). In diphtheria the antitoxin may be given subcutaneously unless the infection is quite severe; in the latter case it should be given intramuscularly or intravenously. In tetanus the serum should be given subdurally and intravenously. In epidemic cerebrospinal meningitis the serum is always given subdurally. In pneumococcus, streptococcus, and gonococcus infections, while the serum may be given subcutaneously or intramuscularly, it is best administered intravenously. *It is important for the physician to know and appreciate that the route and method of inoculation and the amount of serum administered are important factors in determining the success or failure of serum therapy.*

I. TECHNIC OF SUBCUTANEOUS INJECTION

Serum given subcutaneously is slowly absorbed, and a portion of the antibodies may be destroyed before they reach the blood-stream. When large quantities of serum are to be given, as in pneumonia and streptococcus infections, this method may not be permissible on account of the pain and injury to the subcutaneous tissues that may result, aside from the more important question of slow absorption and anchorage or destruction of the antibodies in various tissues before they reach the blood-stream or the focus of disease.

1. Injections should be given where the subcutaneous tissues are loose, where movement is least marked, and preferably where pressure upon the parts is least likely to occur, for some soreness, dependent upon the bulk of the injection, is bound to follow. For these reasons injections may be given in the *abdominal wall*; some prefer the *back*, in the region of the lower angle of one of the *scapulæ*, and the *buttocks*, but in a bedfast patient pressure at these points cannot readily be eliminated.

2. The skin about the site for injection may be prepared by an application of tincture of iodine; this is washed off with alcohol just before the needle is inserted. After the injection has been given the remaining iodine should be removed with alcohol, to prevent the occurrence of a dermatitis, and the puncture wound covered with cotton and collodion or with sterile gauze fastened with adhesive straps.

3. The syringe and needle should be sterile. Manufacturers of biologic supplies furnish antitoxin in syringes ready for injection, and these are usually convenient and satisfactory. The needle should be of medium size, and larger than that used for ordinary hypodermic medication. All glass or glass and metal syringes that may be boiled are to be preferred when a syringe is not furnished. *Before boiling such a syringe the piston should be removed from the barrel, as otherwise it may expand so rapidly as to cause the latter to crack.*

4. When all is in readiness, the syringe being loaded and the air expelled, the skin is pinched up between the fingers and the needle quickly inserted

into the subcutaneous tissues. The injection should be given slowly, and during the operation, if the patient is a child, an assistant should be on hand to prevent struggling. The needle may be connected with the barrel of the syringe by means of a short piece of rubber tubing (Fig. 180). This permits an injection to be given without danger of the needle being broken off if the patient should struggle. Most pain is experienced when the first few drops of fluid are injected; after that the pain is not severe unless the tissues are suddenly distended, as by a quick injection.



FIG. 180.—SUBCUTANEOUS INJECTION OF SERUM.

The site of injection is painted with tincture of iodine and covered with sterile gauze fastened with straps of adhesive plaster. Just before the injection is given the iodine is wiped off with a pledget of cotton and alcohol. A fold of skin is pinched up between the thumb and forefinger of the left hand, the needle inserted, and the serum slowly injected. The needle is then quickly withdrawn, and the puncture covered with the gauze and held in place by the adhesive plaster.

The amount of serum that may be injected in one area depends upon the age of the patient. Due care should be exercised against injecting too much serum in one area, because of slower absorption and possible necrosis of the skin and subcutaneous tissues.

II. TECHNIC OF INTRAMUSCULAR INJECTION

As shown experimentally by Meltzer and Auer, absorption occurs much more quickly when inoculations are given into the muscles than when they are given into the subcutaneous tissues. For this reason antitoxin should be given intramuscularly in severe cases of diphtheria, as the technic is just as simple as that of a subcutaneous injection. Whenever the physician desires more speedy absorption than that which follows a subcutaneous injection, and the intravenous route cannot, for some reason, be adopted, the inoculation should be given in the muscles, preferably those of the *buttocks*.

1. With proper technic very large amounts of fluid even up to 100 c.c. may be injected without unusual pain, deep and superficial infiltrations, abscess formation, or other accident. The object is to introduce the inocu-

lum into the areolar tissue on the upper surface of the fascia forming the extension of the fascia lata covering the gluteus maximus from whence it appears at the lower border of the buttock in the gluteal sulcus with prompt absorption and little or no local irritation.

2. When small amounts are to be injected, as 5 c.c. of milk or serum, 1 c.c. of vaccine, etc., the injections are best given in the same locality and by the technic described, but injections into other muscle groups, as the outer side of the thigh or shoulder, may be employed if the patient is confined to bed by illness.

3. For the gluteal injection the patient should lie prone on a table or bed with muscles relaxed.

4. The site of injection is the lower inner angle of the upper outer quadrant shown in Fig. 181. The skin should be wiped with tincture of iodine and alcohol.



FIG. 181.—INTRAMUSCULAR INJECTION

The lines indicate the quadrants, the injection being given in the lower inner corner of the upper outer quadrant. The left hand has drawn the tissues downward. The needle has been entered and an injection is about to be given.

5. The syringe may be a Luer or Record or some other so made that the needle may be easily detached. The needle should be gage No. 22 and be $1\frac{1}{2}$ inches long for children and very thin adults, 2 inches for the average adult, and $2\frac{1}{2}$ inches for obese individuals. Particular care should be taken to use sharp needles; turned and dulled points and rusty needles are very painful. Syringe and needle should be sterilized and loaded with the inoculum.

6. With the left hand the skin and tissues are drawn downward and flattened; the syringe is grasped with the right hand in such manner that the piston is held against the index-finger and the needle introduced in the direction shown in Fig. 181 by a quick thrust. *The injection should never be made until the operator is sure a vein has not been entered.* For this purpose gently draw on the piston for five to ten seconds; if no blood appears the injection may be given. If the syringe is loaded with defibrinated blood

it is necessary to detach the barrel for a few seconds to determine if blood appears.

7. The injection should be slowly given, the needle *quickly* withdrawn, leakage prevented by massage with a cotton pledget and the wound wiped with alcohol; a small collodion and cotton dressing may be applied.

III. TECHNIC OF INTRAVENOUS INJECTION

The necessity of administering serum intravenously in order to obtain the best results, or any result at all, is becoming more and more apparent. In severe cases of diphtheria the best results are obtained when the anti-toxin is given intravenously; in the treatment of tetanus the tetanus anti-toxin should be administered intravenously as well as intradurally, and both



FIG. 182.—INTRAVENOUS INJECTION.

Air is being expelled from the syringe. This is done by first attaching the needle and expelling air into a pledget of cotton carrying alcohol to collect any fluid that may be expelled.

antistreptococcus and antipneumococcus serums should always be given by the intravenous route. Recent reports indicate that the proper serum treatment of these infections requires large doses given intravenously. Physicians should, therefore, be prepared to give intravenous injections. Since the use of salvarsan in the treatment of syphilis has become so popular many physicians have perfected themselves in the technic of intravenous administration, but there is still great hesitancy about giving intravenous injections, although the methods are relatively simple and easily mastered. With nervous patients the injections can be made practically painless by the preliminary infiltration of the skin about the site of puncture with a few drops of a sterile 1 per cent. solution of eucain or butyn.

Syringe Method.—When small amounts of fluid are to be injected, as

from 1 to 20 c.c., a syringe is employed. The needle should be short and sharp; gage No. 20 is usually satisfactory.

1. It is best to use an all-glass syringe, or at least one with a glass barrel, for the physician can then assure himself that *all air has been expelled and that the fluid is free from solid particles* (Fig. 182). Further than this, a flow of blood into the syringe will indicate that the needle has entered the vein. The syringes furnished by manufacturing firms are not well adapted for making these injections, as the rubber plunger frequently adheres to the glass barrel, so that the injection will be jerky and difficult, and, besides, it may be difficult to determine when the vein has been entered. It is better to empty the contents of these syringes into a large, sterilized, glass-barreled syringe, such as the Record, Luer, and Burroughs-Wellcome syringes, which have a close-fitting but easily working piston, and are attached to the needle by a flange and not by a screw thread. The needle

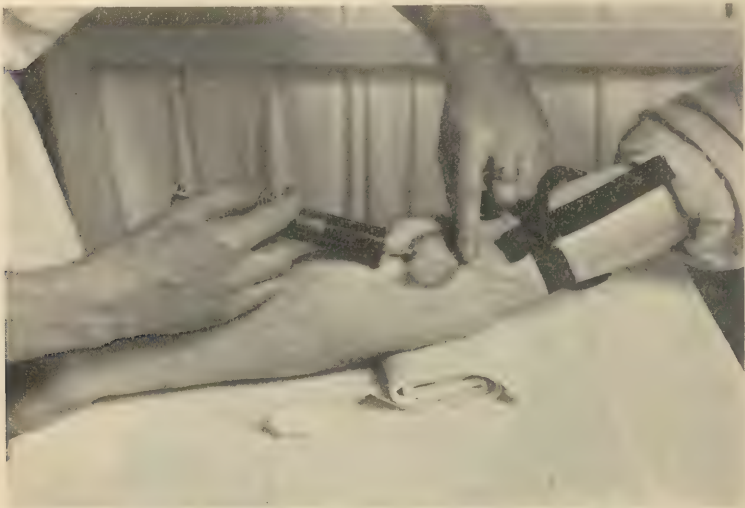


FIG. 183.—METHOD OF MAKING INTRAVENOUS INJECTION BY MEANS OF A SYRINGE.

The vein is being steadied and the needle introduced; gentle suction is then made upon the piston to determine if the needle has been properly introduced, as shown by a flow of blood into the syringe.

should be sufficiently large and have a sharp but short beveled edge. A long point may pierce the vein through and through, and permit perivascular bleeding or result in a subcutaneous injection.

2. In young children with fat arms and a weak circulation it is usually necessary to expose a vein at the elbow by making a small incision. In older children and adults a vein may stand out prominently enough to permit the needle to be inserted directly through the skin without making an incision. A firm rubber tourniquet is applied above the elbow; a very simple one is constructed by a single turn around the arm with a piece of ordinary soft-rubber tubing held in place by a hemostat. After the vein has been entered the tourniquet should be quickly removed and this is quickly and deftly accomplished by releasing the hemostat.

3. The skin about the site of injection is cleansed with soap, water, and alcohol, or merely painted with iodine, which is removed with alcohol just before the injection is to be given, in order that the vein may become visible.

4. An assistant steadies the patient's arm and should be ready to release the tourniquet; or, a slip knot tourniquet of single garter may be employed which the operator may easily release himself as shown in Fig. 183.

5. The operator then steadies the skin over a vein—usually the median basilic or median cephalic—with the left thumb and forefinger, and introduces the needle into the vein (Fig. 183). *A flow of blood into the syringe indicates that the vein has been entered.* The tourniquet is then released and the injection *slowly given.* Or the needle may be detached from the syringe and passed into the vein; when blood appears, the syringe is quickly attached and the injection made. The puncture wound is then sealed with a wisp of sterile cotton and collodion or with gauze and a bandage.



FIG. 184.—METHOD OF MAKING INTRAVENOUS INJECTION BY MEANS OF A SYRINGE.

This syringe was devised for the intravenous administration of serum or a concentrated solution of salvarsan. It is provided with a three-way cock, which permits drawing fluid into the syringe and then injecting it into a vein. This injection may also be given by any glass syringe; the particular advantage of this one is that the operator may inject more than one syringe-full of fluid without removing the needle. The same syringe may be used for the intravenous injection of any serum, as diphtheria and tetanus antitoxins.

The syringe shown in Fig. 184 is well adapted for the intravenous injection of serum, and was devised for the administration of concentrated solutions of salvarsan and neosalvarsan, but any reliable and large glass-barreled syringe may be used.

Gravity Method.—Larger quantities of serum or other fluid (more than 20 c.c.) are better injected by the gravity method, the simple apparatus shown in Fig. 185 being quite satisfactory for the purpose. This consists merely of a graduated cylinder, which serves as a measuring funnel, rubber tubing with a glass window at the lower end and pinch cock, and is furnished with a metal tip that fits the needle. The needle should be of proper size, and have a sharp but somewhat short beveled edge. It may be curved, as shown in the illustration, or may be straight.

1. The cylinder, tubing, and needle should be sterilized by boiling prior to use.

As shown by Stokes and Busman¹ new rubber tubing may cause reactions when employed for the administration of arsphenamin. The same is true regarding the administration of serum and citrated blood. If new tubing is employed it should first be soaked in 5 per cent. sodium hydroxid solution over night and thoroughly rinsed with water and sterilized before use.

The amount of tubing required may be materially reduced by using several windows of glass tubing.

2. The serum or other fluid may be warmed by placing the container in water of a temperature not higher than 42° C. (just comfortably hot to hold the hand in).



FIG. 185.—METHOD OF MAKING INTRAVENOUS INJECTION BY GRAVITY.

This method is suitable for the intravenous administration of salvarsan or antistreptococcus serum, etc. The needle has been entered into a prominent vein (indicated by a flow of blood); the tubing has been attached by means of a metal tip which fits the needle easily and snugly; the tourniquet has been loosened and the injection is being given.

Since serum is viscid and may flow too slowly it is well to dilute it with an equal part of warm sterile saline solution. A further advantage is slower introduction of the serum by reason of its dilution.

3. The injections are best given in a vein at the elbow. The arm about this region should be scrubbed with hot water and soap, followed by alcohol and 1 : 1000 bichlorid of mercury solution, or liberally painted with tincture of iodine. A firm tourniquet is then applied above the elbow; a single firm turn of rubber tubing held by a hemostat is quite satisfactory, as when the vein has been entered the tourniquet should be quickly released with the least movement and disturbance possible, and this arrangement answers all requirements. Sterile towels should be placed about the arm and shoulder.

¹ Jour. Amer. Med. Assoc., 1922, 78, 580.

4. About 20 c.c. or more of sterile distilled water or normal salt solution are then poured into the cylinder, and the cock opened until all air has been expelled from the tubing. The fluid, serum, or salvarsan is then poured into the cylinder. *It is a good practice to filter the fluid through several layers of sterile gauze, especially when salvarsan is being injected, in order to remove any bits of glass or other foreign bodies that may be present.*

5. An assistant holds the loaded cylinder and tubing; the operator steadies the skin over a prominent vein and quickly inserts the needle. A flow of blood indicates that the vein has been penetrated. The tubing is then quickly and carefully attached, the tourniquet released by unfastening the hemostat, and the injection *slowly given*. As a rule, an elevation of the

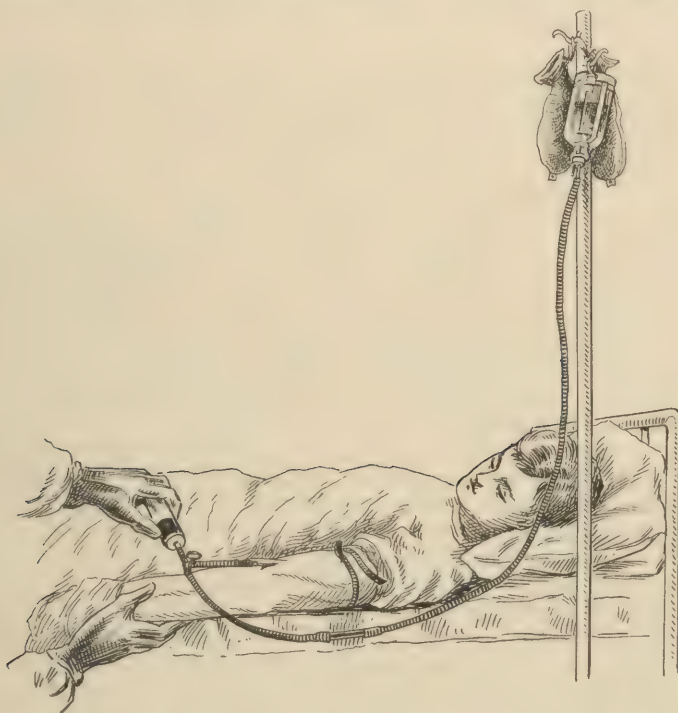


FIG. 186.—APPARATUS FOR THE INTRAVENOUS INJECTION OF SERUM (ROCKEFELLER HOSPITAL).

The diluted serum is kept warm by means of two water bottles (left apart in the illustration to show the container). The syringe is attached with a two-way cock for the purpose of filling and then slowly injecting the serum into the patient.

cylinder of 2 or 3 feet is sufficient. If swelling occurs about the site of puncture and the patient complains of pain, the injection is entering the subcutaneous tissue; when this occurs, the pinch-cock should be closed and the needle removed. It is then necessary to make the injection into another vein or into the same vein at another site.

6. When serum is being given, as antipneumococcus serum for example, the injection of the first 10 to 15 c.c. should occupy ten to fifteen minutes. During this time the patient should be carefully watched for pallor, cyanosis, dyspnea, and tachycardia. If these symptoms appear, it is well to stop the injection for ten or fifteen minutes to see if they increase in severity. Usually they disappear, in which case the injection is resumed. As a general

rule no symptoms appear and the injection (100 c.c. serum and 100 c.c. of saline) can be given in ten to fifteen minutes.

7. Figure 186 shows the apparatus employed in the Rockefeller Hospital. By means of the syringe the serum is aspirated from the container surrounded by hot-water bags to keep the solution warm, and injected into the vein. This arrangement is particularly serviceable for the injection of the first 15 c.c. at the rate of 1 c.c. per minute and especially if the patient is suspected as being hypersensitive.

Intravenous Injection of Children.—This is usually much more difficult than with adults. Children over three years of age, however, frequently present a sufficiently prominent vein at the elbow for successful intravenous injections and especially when a syringe is employed with a No. 22 needle. Otherwise the skin may be infiltrated with sterile eucaïn solution and a vein exposed.

The external jugular vein may be employed as shown in Fig. 18.

In infants the injections are best given by way of the superior longitudinal sinus, the technic being described in Chapter II and illustrated in Figs. 21–23.

TECHNIC OF INTRASPINAL INJECTION

In the treatment of epidemic cerebrospinal meningitis, influenzal meningitis and tetanus, the specific serums are administered subdurally by means of a needle introduced in the lumbar region. Every practitioner should be prepared to perform lumbar puncture for the purpose of securing cerebrospinal fluid for making the Wassermann reaction and the bacteriologic, cytologic, and chemical examinations, and the administration of serum is a relatively simple matter when the puncture has been successfully made.

The technic of lumbar puncture for the purpose of securing fluid for diagnosis is described on p. 25. But when administering serum, and especially in the treatment of meningitis, the clinical condition of the patient and the danger of sudden collapse render it advisable and necessary that the inoculation be given with the patient lying on his side.

Methods.—Two methods are now being employed. The older method consists in injecting the serum by means of a *syringe*, and the later one is a method whereby the serum is allowed to flow in by *gravity*.

Not infrequently a patient will develop symptoms of collapse during a subdural injection, and these have been ascribed to undue pressure, the injurious action of trikresol or other preservative upon the respiratory centers, too rapid injection, and the introduction of too large a quantity of serum. It is now apparent that in the past too little attention has been paid to the patient while the injection was being made, and serum has usually been administered according to more or less fixed and arbitrary rules, instead of being guided by the clinical condition of the patient.

If symptoms of collapse appear during a subdural injection, they may be relieved by allowing the fluid within the canal to flow out again, and this is best accomplished when the inoculation is given by the gravity method. The latter method has been recommended by Sophian, Flexner, and the Hygienic Laboratory, and is undoubtedly the method of choice.

Blood-pressure as a Guide in Administering Serum Subdurally.—According to the older and customary method of injecting serum subdurally, fluid is permitted to flow from the needle until from 15 to 20 c.c. have been removed, and an equal quantity of serum is then injected. In severe cases, with thick plastic exudates, only a few cubic centimeters of fluid may be withdrawn, and, indeed, no fluid at all may be secured. To inject arbitrarily

a fixed amount of serum under such conditions may be highly dangerous to the patient on account of increased pressure. On the other hand, when the flow is free, it may be dangerous to permit the canal to drain until intraspinal pressure is reduced to the normal, a fact indicated by the flow of a drop of fluid every three to five seconds.

With these considerations in mind, Sophian¹ has studied the value of cerebrospinal fluid pressure and blood-pressure as controls on the amount of fluid that may be safely withdrawn and on the amount of serum that may be injected. During the study and treatment of 500 cases of epidemic cerebrospinal meningitis this last-named observer found that the blood-pressure was a valuable guide. Cerebrospinal fluid pressure was found to be misleading, owing probably to a local distention of the subarachnoid space at the site of injection, which resulted in readings that did not represent the true intracranial pressure.

1. Usually upon the withdrawal of cerebrospinal fluid a fall of blood-pressure occurs. With the ordinary blood-pressure in an adult patient—about 110 mm. of mercury—Sophian recommends stopping the flow when there has been a drop in pressure of about 10 mm. of mercury; in children, about 5 mm. In a few cases there is no change in blood-pressure or even a slight rise; in these instances fluid may be removed until the flow has diminished to the rate of a drop every three to five seconds.

2. With the injection of serum the blood-pressure drops still further. Generally the decrease in blood-pressure is proportional to the rapidity with which the serum is injected and the amount injected. By the gravity method, under ordinary conditions, at least ten minutes should be consumed in administering 15 c.c. of serum. A total drop of 20 mm. of mercury indicates that sufficient serum has been injected. If it is desired to inject more, as in a severe case of meningitis, close watch should be kept for other symptoms of collapse.

3. Usually, under these conditions, less serum is administered than has been advocated heretofore. It is apparent that the more potent the serum, the less bulk is required—and the bulk alone is an important factor, for a large injection may so injure the patient as to counteract any good that the serum may do. Unfortunately, there is no accurate measure of the curative value of antimeningococci serum. It is highly desirable that a serum be as potent as possible, and the physician must rely upon the reputation of the firm producing the serum. Efforts are being made to concentrate these serums, much as antitoxin is concentrated, and this is an end very much to be desired.

4. Blood-pressure changes are not constant in the same patient upon different occasions. The pressure should be taken after each puncture and inoculation, for the administration cannot be guided by observations made on a previous occasion.

Collapse During Subdural Inoculation.—Carter has shown, by experiments on dogs, that the first mechanical effects of increased intraspinal pressure were respiratory depression and marked cardiac inhibition. Sophian has found that similar effects may be produced during subdural injections of serum in the treatment of meningitis.

The *symptoms* of collapse, such as stupor, superficial or deep, irregular and slow respiration, and dilatation of the pupils, are foreshadowed by a marked drop in blood-pressure. The pulse may continue good or become slow and irregular. Incontinence of urine and feces may occur.

The *treatment* consists primarily in discontinuing the injection. By

¹Epidemic Cerebrospinal Meningitis, 1913, Mosby Co., St. Louis.

lowering the funnel fluid is allowed to flow from the spinal canal and mix with the serum. If a syringe is being used, it should be detached from the needle or gentle suction made. After a few minutes the symptoms may disappear and the inoculation may be cautiously resumed until the desired amount of serum has been injected; otherwise the needle should be withdrawn.

In addition to this procedure atropin and caffein may be administered hypodermically in large doses, and artificial respiration resorted to if necessary. It is well to have these drugs ready for injection before the inoculation is begun, so that no time will be lost when they are needed.

Anesthesia for Subdural Inoculation.—There is no doubt that lumbar puncture and the subdural injection of fluid are painful, the amount of pain depending to some extent upon the degree of meningitis, the method of injection, and the skill of the operator. Severe cases of meningitis that are stuporous or moribund may not evince any evidences of added discomfort; less toxic and robust or nervous patients may, however, suffer considerably and prove difficult subjects for injection.

Local anesthesia may be secured by injecting a sterile 1 per cent. solution of eucaïn in the region where the puncture is to be made (see Fig. 24). General anesthesia for lumbar puncture in meningitis adds a considerable element of danger, but if it is absolutely necessary, a few whiffs of ether or chloroform may be given while the needle is being inserted. In giving subdural injections in tetanus a general anesthetic is necessary.

Sophian has found that if water is given through a straw while performing lumbar puncture patients will frequently drink large quantities of it and keep very quiet.

Gravity Method.—The *apparatus* required is very simple, and consists essentially of a proper needle and from 12 to 16 inches of soft-rubber tubing attached to a container or funnel for serum and furnished with a metal tip by which it is quickly and readily attached to the needle.

Several manufacturers of biologic supplies are marketing antimeningococcic serum in a special container, fashioned after that devised by Sophian and Alexander, with the needle and tubing adapted for the administration of the serum by the gravity method. Such an apparatus is shown in Fig. 187.

The physician may, however, prepare an equally efficient apparatus, similar to that shown in Fig. 188, which consists of the glass barrel of a 20 c.c. syringe attached to 14 inches of soft-rubber tubing fitted with a metal tip that holds the needle firmly and snugly. The whole is sterilized by boiling, and any quantity of serum may be administered with it. The apparatus is adapted for the administration of antimeningococcic serum, tetanus antitoxin, influenza serum, salvarsanized serum, or any other fluid, and has given uniform satisfaction.

The needle should be from 10 to 11 cm. in length, with a wide, rather than a narrow, lumen—about 1.5 to 2 mm. This is important in administering serum to a case of meningitis, in which a needle with a narrow lumen may become plugged with exudate. The needle should be fitted with a trocar. The tip should have a short bevel with a sharp edge.

Technic.—1. The serum should be warmed to body temperature by wrapping the sealed container of serum in towels wrung out of water comfortably hot for the hands (about 42° C.). Cold serum possibly increases the pain caused by the injection, although the pressure upon the sensitive nerve-roots is the chief source of pain and discomfort. Due care must be exercised that the serum is not coagulated by too high temperature.

2. The funnel or syringe barrel, tubing, and needle should be sterilized

by boiling. The outfits furnished by the manufacturers are sterilized and ready for use. Two sterile *graduated* centrifuge tubes should be on hand for collecting and measuring the spinal fluid. The apparatus should be assembled and ready for injection, so that at the appointed time the tubing may be attached to the needle and the inoculation given.



FIG. 187.—OUTFIT FOR INTRASPINAL INJECTION OF ANTIMENINGITIS SERUM BY GRAVITY (Sophian)

3. The patient should be placed on the left side, on the edge of a bed or table. An assistant places the patient in such a manner as to arch the back as much as possible. A second assistant takes blood-pressure readings during the operation (Fig. 188).

4. Towels wet with bichlorid are arranged about the site of inoculation. It is well for the operator to locate the site of injection by palpating the spinous processes and selecting the widest interspace, which is usually on a level with the crests of the ilia if the back is well arched. The skin is then

cleansed with soap, water, and alcohol, and bichlorid solution or a coat of iodine applied. Some degree of local anesthesia may be secured by injecting a small amount of a sterile 1 per cent. solution of eucain. This is advisable in nervous adults (Fig. 24).

The usual *site of puncture* is between the fourth and fifth lumbar vertebræ, but in repeated punctures it may be necessary to puncture one or two interspaces higher up in order to avoid pain and the formation of adhesions in the subarachnoid space.

Lumbar puncture may be done as high as between the first and second lumbar vertebræ, and when no fluid is obtainable at the lower levels, high puncture must be conducted. Punctures may also be done between the twelfth dorsal and first lumbar vertebræ and even between the eleventh and



FIG. 188.—INTRASPINAL INJECTION BY GRAVITY.

The line marks the crest of the ilium, and indicates the third lumbar interspace. The needle has been inserted and cerebrospinal fluid withdrawn. Antimeningococcus serum is being administered. The barrel of a 20 c.c. *Record* syringe is serving as a funnel, and is attached to the needle by means of 14 inches of soft-rubber tubing furnished with a metal tip. The needle, as shown, is reduced to about four times its actual size. This method may be used for making the subdural injection of tetanus antitoxin² and salvarsanized serum.

twelfth dorsals. Above the level of eleventh dorsal puncture may be useless because of an incomplete posterior subarachnoid space in many individuals at this level, but puncture may be done high in the dorsal or at the lower end of the cervical region and the canal washed out with sterile saline solution between this point and a needle in the lower lumbar region.

However, *high punctures are more dangerous than low punctures by reason of possible injury to the spinal cord.* For this reason punctures above the tip of the cauda equina (third lumbar vertebra in infants and first lumbar vertebra in adults) must be made with extraordinary care.

5. The operator must then choose between the median or lateral route of puncture. The median is the easier, and should always be adopted by the inexperienced operator.

Wash off the iodine with a pledget of cotton soaked in alcohol. Locate the chosen interspinous space, pressing well between the spines with the left thumb or index-finger, and holding the finger in place pass the needle perpendicularly in the median line between the spines, or, better still, at an angle of 45 degrees upward and inward. If an obstruction is felt, withdraw the needle slightly and pass it in a different direction until it imparts a sense of "giving way," which indicates that the subarachnoid space has been reached. Quincke has estimated the depth of lumbar puncture in adults to be usually from 4 to 6 cm.; in large muscular men it is from 7 to 8 cm., and in fat persons, about 10 cm.

The needle should be inserted slowly and deliberately, rather than quickly, as puncture of a bone is likely to be followed by a dull, aching pain, and, indeed, the point of the needle may be bent or broken.

The fluid may fail to flow or flow very slowly. This may be due to the presence of a thick exudate, impalement of a nerve filament, or adhesions arising from a previous puncture. The needle may be turned gently or the trocar inserted to remove an obstruction, after which the flow usually starts; if it does not do so, the needle may be withdrawn slightly or cautiously inserted a little further.

6. Fluid is collected in the centrifuge tubes while blood-pressure readings are being made. When the pressure drops 10 mm., or if the flow is about a drop every three or five seconds, the tubing is connected and the serum injected very slowly.

As a general rule, as much fluid should be withdrawn as can be done with safety, and the maximum dose of serum given. When the flow is scanty, a larger dose of serum may be given than counterbalances the fluid removed, the injection being guided by the blood-pressure. When the total drop reaches 20 mm. of mercury, the injection should be discontinued, or, if continued, the patient should be watched closely for other symptoms of collapse.

7. After the injection has been completed the needle is quickly withdrawn and the wound covered with sterile gauze held in place by adhesive straps. All iodine should be washed off with alcohol to avoid irritation or an actual dermatitis.

Syringe Method.—1. The technic is practically the same as that just described, except that the injection is given with a syringe.

Manufacturing concerns market their products in syringes all ready for injection, but at the present time the gravity method is used almost exclusively. When injecting tetanus antitoxin it is necessary to empty the syringe into another sterile syringe, as shown in the accompanying illustration (Fig. 189), which will fit an appropriate needle. Since the plunger of the purchased syringe oftentimes adheres to the barrel and renders the injection jerky and difficult, I frequently transfer the serum to a sterile, all-glass syringe which I know will work smoothly and satisfactorily.

2. Lumbar puncture is performed as for the gravity method while blood-pressure observations are being made. When sufficient fluid has been removed, the loaded syringe is attached to the needle and the injection *slowly given*. The physician is frequently tempted to inject the serum and complete the operation quickly, but it is better to inject it in amounts of 0.5 to 1 c.c. every half to one minute, being guided by the blood-pressure readings and general condition of the patient. If the pressure falls below 20 mm. of mercury or other symptoms of collapse appear, the fluid may be drained from the canal by *gentle* suction with the piston. This is usually impossible when the manufacturers' syringe is used. Otherwise the syringe

is detached and the fluid collected in tubes until the patient's condition improves and the injection is resumed or the needle removed.

At times the patient is so restless that this slow method is not feasible. In such instances the physician should make the injection as slowly as possible, endeavoring to put into the canal as much serum as fluid was removed or at least a reasonable amount.

Difficulties in Lumbar Puncture.—*In failure to enter the subarachnoid space* it is well to withdraw the needle entirely and try again, rather than withdrawing part way and probing.

Obstruction of the needle may be due to impaction with a nerve root, in which case rotation of the needle suffices. The needle may not be introduced far enough, a part of the eye being outside of the arachnoid space, or



FIG. 139.—INTRASPINAL INJECTION BY MEANS OF A SYRINGE.

The line indicates the crest of the ilium, and usually passes between the third and fourth lumbar vertebrae, which is the proper point for inserting the needle. The site of injection has been painted with tincture of iodine after cleansing with soap, hot water, and alcohol. An assistant holds the patient to prevent sudden jerking and possible accident.

it may be introduced too far. The needle, therefore, may be gently introduced or withdrawn to a slight extent. A flake of pus may obstruct; careful introduction of the stylet and rotation suffices for correction.

Dry tap may be due not only to these causes, but likewise to hydrocephalus, either internal by obstruction of the foramina of Magendie and Luschka, or external, the upper part of the subarachnoid space being cut off by adhesions.

Hemorrhage may occur due to puncture of a vein somewhere between the skin and subarachnoid space. In this case only the first few cubic centimeters of fluid are stained.

A vein belonging to the plexus lying on the ventral aspect of the spinal cavity may be punctured when the needle has been introduced too far; this is indicated by more copious and continuous bleeding.

Blood may appear at the end of the flow due to the rupture of a venule as a result of injury or lowering of intraspinal pressure.

The fluid may be uniformly hemorrhagic due to the extreme intensity of the inflammation; in this case the fluid does not clot. Cases of this kind have a very unfavorable prognosis.

Sequelæ of Lumbar Puncture.—There is usually a drop of temperature within four to eight hours due to the withdrawal of the purulent fluid and regardless of whether or not serum has been given.

Sometimes a nerve root is struck during puncture, producing pain in the legs or pelvis, but paralyzes of the bladder, rectum, or of muscle group or paraplegia are very rare. Usually the patient complains of pain during the injection of serum owing to pressure, but after the injection is completed this distress rapidly disappears. Pain and weakness in the back, so often complained of for some considerable time after recovery from meningitis, is more probably a sequel of the disease itself than of repeated lumbar puncture (Worster-Drought and Kennedy).

Serum sickness develops in many cases and the physician should be perfectly familiar with the symptoms and warn the family of its possible occurrence in order to allay fears of recrudescence of the meningitis.

METHODS OF PREPARING HUMAN SERUM FOR AUTOSERUM AND CONVALESCENT SERUM INJECTIONS

As will be discussed shortly in Chapter XL human convalescent serum and blood have proved of value in the treatment of scarlet fever, acute anterior poliomyelitis, and some other diseases; injections of the individual's own serum (autosserum) have been found useful in the treatment of some skin diseases and for intraspinal injection in the treatment of neurosyphilis according to the Swift-Ellis method. Normal human serum and blood are also employed for the treatment of hemorrhage.

Method for Securing Small Amounts of Human Serum.—1. When only 5 to 10 c.c. of serum are to be given, 25 c.c. of blood may be aspirated from a vein at the elbow with a Luer or Record syringe and expelled into a sterile centrifuge tube or a test-tube that may be centrifuged. The skin over the vein should be cleansed with tincture of iodine and alcohol and every step carefully guarded against contamination.

The serum may be allowed to separate spontaneously (usually over night) or after coagulation has occurred the clot may be gently broken up with a sterile glass rod and the serum secured within a few hours by centrifuging. The supernatant serum is then carefully pipetted into a sterile test-tube.

2. For larger amounts of serum, as 15 to 20 c.c., blood may be secured from a vein at the elbow into a special large centrifuge tube shown in Fig. 190. This tube easily carries 50 c.c. of blood and fits the International centrifuge. The neck is narrowed so that a rubber stopper may be employed when centrifuging. Osborne¹ has recently described a very convenient type of stopper, but the ordinary rubber stopper suffices. A cotton or gauze stopper may be used during the collection of the blood, but is not adapted for centrifuging because it may be driven into the tube unless fastened with rubber bands, and even then fibers may be driven into the blood, increasing the risks of contamination.

It is essential to use a sharp, short, and rather wide bored needle. That shown in Fig. 190 is gage 18, 1 inch in length, and fits the Record syringe. To the end is attached 3 inches of soft-rubber tubing, the free end being

¹ Jour. Amer. Med. Assoc., 1922, 78, 580.

in the centrifuge tube, and the needle enclosed in a small test-tube strapped to the centrifuge tube by means of rubber bands.

3. Blood is usually secured from a vein at the elbow a little below the crease in order to minimize the amount of discomfort during the following day or two. A firm tourniquet is applied and the patient requested to open and close the hand rapidly and vigorously which aids in distending the superficial veins. The skin is cleansed and tincture of iodine applied, which is later wiped away with alcohol in order that the vein may be seen.

The needle is removed from the test-tube and grasped with a hemostat in the right hand, avoiding contamination of the tip; the tube is held in the palm of the hand and the needle inserted into the vein (Fig. 191). The patient is requested to quietly open and close the hand, which increases the flow of blood and prevents coagulation in the needle and tubing. After securing the desired amount of blood the *tourniquet is released before the needle is removed* in order to prevent bleeding

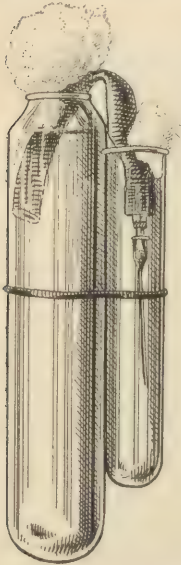


FIG. 190.—APPARATUS EMPLOYED BY AUTHOR FOR ASEPTIC COLLECTION OF SMALL AMOUNTS OF BLOOD.

Consists of a 50-c.c. centrifuge tube fitted with a short piece of tubing and No. 18 needle (length 1 inch). Needle enclosed in a test-tube and the whole sterilized by autoclaving.



FIG. 191.—COLLECTION OF BLOOD.

This method is especially useful for the collection of blood for auto-serum treatment.

into the tissues. The needle and attached tubing are replaced in the test-tube.

4. The blood is allowed to coagulate at room temperature and then placed in a refrigerator for the separation of serum; or after coagulation has taken place the clot may be broken up very thoroughly with a sterile glass rod, the rubber stopper sterilized by boiling for a few minutes, and the serum secured at once by centrifuging.

5. The supernatant serum is now carefully removed with a sterile pipet into a sterile test-tube ready for immediate injection. If serum is to be kept for some time it is well to add 0.5 c.c. of 5 per cent. solution of phenol or tricresol to each 10 c.c. of serum (0.25 per cent.) with storage in a refrigerator.

Method of Securing Large Amounts of Human Serum.—For securing blood from scarlet fever or poliomyelitis convalescents the above method may be employed when only small amounts of blood are to be taken.

As a general rule, however, larger amounts up to 200 to 500 c.c. are usually withdrawn from adult subjects in good condition.

Since the serum is to be used for the treatment of other individuals reasonable care should be exercised against the selection of syphilitic and tuberculous subjects. After the serum has been secured a small amount should be reserved for the Wassermann test and 5 c.c. planted in a flask of glucose broth for culture.

The technic for collection is the same as described above except that a larger container is used and means provided for facilitating the flow of blood and preventing coagulation in the needle and tubing by creating a partial vacuum in the container. The apparatus shown in Fig. 192 is

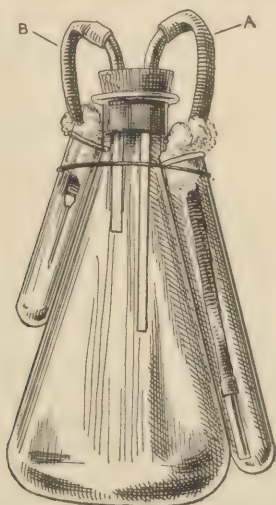


FIG. 192.—APPARATUS FOR ASEPTIC COLLECTION OF LARGE AMOUNTS OF BLOOD.

Tube *A* is for suction; tube *B* is for collection of blood.

convenient and readily assembled. It consists of a 500-c.c. Erlenmeyer flask with a two-holed rubber stopper fitted with two pieces of glass tubing for attaching the needle and suction tube, the whole being sterilized by autoclaving.

After blood has been collected the stopper may be replaced by a sterile solid rubber or gauze and cotton stopper. If separation of the serum is incomplete or a portion becomes bloody, the material may be removed to centrifuge tubes with sterile pipets and clear serum secured by centrifuging.

Each serum should be cultured for bacteria and tested for syphilis as mentioned above. If these tests are satisfactory the sera of several individuals may be mixed, preserved with 0.25 per cent. phenol or tricresol, and stored in a refrigerator in 20-c.c. bottles.

CHAPTER XXXVIII

PROPHYLACTIC SERUM OR PASSIVE IMMUNIZATION

SERUM PROPHYLAXIS OF DIPHTHERIA

Immunity in Diphtheria.—Diphtheria is a disease almost entirely confined to the human race. Cats have been known to suffer with it and act as a source of infection for children. Several other of the lower animals, as rabbits, dogs, birds, and notably the guinea-pig, are susceptible to injections of the bacilli or toxins, but do not contract the disease spontaneously.

Not all human beings are susceptible. Since the advent of the Schick test it is now known that some may possess in their blood sufficient natural antitoxin to protect against diphtheria, the minimum required amount being at least 1/30th unit per cubic centimeter. Children under six months of age are generally immune by reason of congenital antitoxic immunity conferred by the mother. After six to eight months this immunity disappears from the majority of children. The disease is most common between one and seven years of age. After ten to twelve years a natural immunity develops in many individuals and particularly in the crowded districts of cities; this is probably due to the production of antitoxin as the result of numerous slight infections. The majority (60 to 70 per cent.) of adults are naturally immune. All races are susceptible; the disease is somewhat more common among boys than girls, probably owing to the greater danger of contact infection.

One attack of diphtheria does not always confer a lasting immunity; second and even third attacks are not uncommon. This is probably because our body cells respond slowly to stimulation by diphtheria toxin with the production of antitoxin, and the ordinary attack of diphtheria is too brief in duration for adequate stimulation of antitoxin production. This is indicated by the fact that several months are required for the production of sufficient amounts of antitoxin after the injection of toxin—antitoxin mixtures.

Toxins are the most important pathogenic agents in diphtheria and antitoxin is the most important antibody. Probably other immunity principles are also concerned both in affording protection and in recovery—especially phagocytosis. Bacteriolysins and opsonins are sometimes demonstrable during diphtheria and probably contribute to the mechanism of recovery.

Serum Prophylaxis.—The subcutaneous administration of relatively small doses of antitoxin will usually confer a passive immunity against diphtheria lasting from two to four weeks.

The *object* is to introduce antibodies (antitoxin and opsonin) into the body fluids in order that they may neutralize the toxin as rapidly as it is produced, aid in the destruction of the bacilli, and thus protect the individual in case virulent bacilli should be inspired or otherwise gain access to the tissues.

Dosage and Administration.—The *doses* advised are relatively small, and the injection does not usually produce any discomfort other than soreness about the site of inoculation. For infants under one year of age 500 units suffice; for older children and adults from 1000 to 1500 units should be given. Antitoxin has been administered orally, but the results are

irregular and the degree of immunization inconstant; for these reasons the serum should be given by subcutaneous injection.

Duration of the Immunity.—The *duration of this passive immunity* is relatively short, owing to the fact that the antitoxin is eliminated rapidly, as it is part and parcel of a foreign serum that tends to be excreted or destroyed soon after its introduction into the body. It will endure, however, for at least two weeks, and frequently two to four weeks longer. Since the incubation period of diphtheria is only a matter of a few days, this suffices, in the majority of instances, to protect the individual. But if the danger of infection lasts for more than six weeks a second injection may be required.

Indications.—The *indications* are to immunize all persons who have come in intimate contact with a case of diphtheria. *If time permits the Schick test should be conducted, as only those persons yielding positive reactions require the antitoxin* (see p. 739). It is especially valuable in families and small communities, such as go to make up hospital wards and asylums. The physician who is attending a case of diphtheria in a private home should urge immunization upon all members of the household who have been exposed to infection.

The Schick Test in Relation to Passive Immunization Against Diphtheria.—As stated above only those persons showing a positive Schick reaction require immunization when exposed to diphtheria because of susceptibility to the disease. Children over two years and adults who have yielded negative Schick tests *properly conducted* do not require immunization because they are immune to the disease probably for life. For this reason the Schick test is particularly valuable to physicians, nurses, and others especially likely to be exposed to diphtheria; if known to be Schick negative the administration of antitoxin is not required.

When time permits the Schick test may be conducted before antitoxin is injected on a large scale to *adults*, as inmates of a ward or institution; this delays the injection of antitoxin, but in my opinion is justifiable as the majority of adults are immune and will yield negative reactions. With *children*, however, from two to twelve years of age this delay may not be advisable if there is reason to believe that they have been intimately exposed, because the majority are susceptible to the disease and yield positive Schick reactions.

Results.—The *immediate results* are usually good. The main disadvantage is the short duration of the immunity, so that no matter how faithfully it is carried out, persons do not remain immune for long periods of time, and accordingly the total morbidity of the disease is not influenced to any extent. In homes from which the case of diphtheria is promptly removed to a special contagious hospital and in which the remaining members are promptly immunized the percentage of secondary cases is practically nil. Of 6772 patients who were removed to the Philadelphia Hospital for Contagious Diseases, the remaining members of the family not being immunized, secondary cases developed in 164 persons, or in 2.4 per cent. Of 4063 cases of diphtheria treated at home with antitoxin, the other members of the family not being immunized, secondary cases developed in 219 persons, or in 5.3 per cent. Of 639 diphtheric patients treated at home who did not receive antitoxin and where immunization was not practised, secondary cases developed in 151 persons, or 23.6 per cent. These figures, compiled by Dr. A. A. Cairns, chief medical inspector of Philadelphia, and taken from the annual reports of the Philadelphia Bureau of Health for the years of 1909, 1910, and 1911, show that the best results are obtained when the

diphtheric patient is promptly removed to a special hospital and the remaining members of the household are immunized. Similar results have been reported by Braun,¹ who found that only 1.62 per cent. of a group of 2218 children exposed to diphtheria and immunized with serum developed the disease, whereas nearly 35 per cent. developed diphtheria in families not immunized. Even when the patient is removed promptly there is some danger of other persons having been infected, and immunization should, therefore, always be promptly practised. When the patient is treated at home, other members of the household, even if immunized, are liable to develop the infection, probably owing to the fact that the patient harbors virulent bacilli for varying periods of time after the passive immunity in other persons has passed away and the quarantine is broken. Certainly in those homes where antitoxin is not used either for therapeutic or for prophylactic purposes, the percentage of secondary infections is so high as to leave no doubt as to the value of antitoxin.

In this connection I may mention the desirability of using an antitoxin prepared by immunization of cattle for the general purpose of prophylaxis, and especially for the treatment of those persons who are hypersensitive to horse serum. In these cases horse antitoxin could be used later if a person contracted diphtheria without danger of anaphylaxis.

VON BEHRING METHOD OF TOXIN-ANTITOXIN VACCINATION AGAINST DIPHTHERIA (T-A IMMUNIZATION)

Historical.—Owing to the fact that the antibodies produced through the activities of our own body cells (active immunization) persist for longer periods of time than those that are introduced passively (passive immunization), von Behring and his assistants have developed a method of active immunization in diphtheria whereby our own body cells are stimulated to produce our own antitoxin in sufficient amounts to protect us against the disease. It has long been known that more or less balanced mixtures of this kind produce immunity in animals. Babes in 1895 was the first to inject experimentally diphtheria toxin-antitoxin mixtures and to appreciate that not only slightly toxic but also that slightly overneutralized mixtures would engender the production of antitoxin in animals. At about the same time and independently, Park of New York made the same observation and began to use this knowledge in the immunization of horses for the production of antitoxin. Park noted that the use of large amounts of overneutralized toxin was a safe and rapid method of beginning immunization of horses; that is, the antitoxin protected against fatal reactions, but yet active immunization occurred because small amounts of toxin became gradually dissociated and served to stimulate the body cells to produce antitoxin. Exactly the same mechanism is operative in the immunization of human beings with these T-A mixtures.

In 1907 Theobald Smith² suggested that it might be possible to employ this method for the purpose of producing immunity in man. Subsequently Smith³ studied the effects of injections of neutral mixtures in guinea-pigs and horses, and again pointed out the applicability of the method to human beings. Finally this was accomplished by von Behring⁴ in 1913, by demonstrating in a few human beings the safety of the injections. Shortly after-

¹ Deutsch. med. Wchn., 1913, 40, 1145.

² Jour. Med. Research, 1907, xvi, 359.

³ Jour. Exper. Med., 1909, xi, 241; Jour. Med. Research, 1910, xxiii, 433.

⁴ Deutsch. med. Wchn., 1913, 39, 873

ward Hahn and Summer¹ injected 1097 children in the district of Magdeburg, where diphtheria was endemic. Of these, 633 received the full series of three injections, 255 received two injections, and 209 received one. The Schick test was not employed. For the first two weeks there was no difference in the number of cases of diphtheria in the vaccinated and unvaccinated, but after that time there was a lessening of the number in the vaccinated group. In 1920 Bieber was able to study the histories of the majority of these individuals and reported that during the six years, 15 per cent. of the unvaccinated group contracted diphtheria, whereas only 4.6 per cent. of the vaccinated contracted the disease.

Mechanism of Toxin-antitoxin Immunization.—Active immunization in diphtheria could probably be accomplished by the administration of minute and increasing doses of toxin, but there would be some danger of producing local necroses, an acute toxemia or paralysis, and the process may require so much time as to be useless in the presence of epidemics.

von Behring's method, according to his report read before the German Convention on International Medicine in 1913, is based upon the principle that the union of toxin and antitoxin is not stable, and when a neutral mixture of the two is injected into animals, sufficient toxin becomes dissociated to unite with body cells and stimulate the production of antitoxin.

The amount of antitoxin injected does not confer an appreciable immunity; therefore this method of immunization cannot be regarded as a combined active and passive immunization, but is purely a process of vaccination or active immunization by means of the toxin stimulating our body cells to produce antitoxin. For convenience only the subject is here considered instead of in the chapter on Active Immunization.

Preparation of the Toxin-antitoxin.—Formerly immunization was conducted with mixtures of toxin-antitoxin which were slightly overneutralized or just neutral; at the present time mixtures slightly toxic for the guinea-pig are being employed. According to Zingher² these may be prepared as follows:

"Only a well-ripened toxin with an L+ dose of 0.4 to 0.2 c.c. should be employed. Concentrated antitoxin of carefully determined strength is added in such proportions that 85 per cent. of each L+ dose of toxin is neutralized by one unit of antitoxin. The actual amount of antitoxin for each L+ dose of toxin is 1.17 units. For example, a toxin with an L+ dose of 0.4 c.c. contains 2.5 L+ doses per cubic centimeter and will require 2.5×1.17 or 2.9 units of antitoxin to each cubic centimeter or 2900 units for the liter of toxin."

The mixture is thoroughly shaken, allowed to stand for two hours, and sterility and toxicity tests conducted. For the latter purpose a guinea-pig is injected subcutaneously with 1 c.c. of the mixture and should develop only a slight induration; a second pig is injected with 5 c.c. and should show a moderate or marked local edema and late paralysis, but should not die of acute poisoning. If the animal dies before the fifth day, it indicates an excess of free toxin in the mixture which has to be carefully neutralized. The mixtures should be kept cold in a refrigerator and are effective for at least three months. As the mixture stands the toxin tends to deteriorate faster than the antitoxin and for this reason perfectly fresh mixtures are better. This, however, is not practical and not necessary if well ripened toxin and antitoxin are employed.

The mixtures as described contain from 2 to 3 L+ doses of toxin in each

¹ *Deutsch. med. Wchn.*, 1914, 40, 13.

² *Jour. Infect. Dis.*, 1917, 21, 493.

dose, properly neutralized. In older children and adults they sometimes produce severe reactions, and recently Park has been using in each dose only 1/10 L+ dose of toxin, properly neutralized. Preliminary results indicate that the antitoxin production is just about as good, and that these severe reactions are avoided.

Dose and Method of Administration.—The dose is 1 c.c. injected subcutaneously once a week until three doses have been given. For children under one year of age three injections of 0.5 c.c. each are given. The injections may be given in the arm at the insertion of the deltoid muscle or under the skin of the abdomen. Zingher has found that two injections of larger amounts (1.5 c.c.) do not give as good results as three injections of a smaller amount. As previously stated, the mixture should be underneutralized, but is perfectly safe for the human being. In about 5 to 8 per cent. of Schick positive individuals a fourth and even a fifth dose may be required to render them immune (Schick negative).

Age in Relation to Immunization.—Apparently the most favorable age is from six months to two years. According to Park¹ infants under six months do not usually respond with the production of antitoxin because of the combined effect of immature cells and the overneutralization of the toxin-antitoxin mixture (due to the presence of natural antitoxin in the blood); the injections, however, produce no ill effects.

As previously stated, it is scarcely worth while conducting a preliminary Schick test with children six months to two years of age because if a negative reaction occurs this result may be temporary and misleading and the child lose its immunity later on as the natural antitoxin disappears. Toxin-antitoxin may be given to all, even though it means that a few children may not actually be in need of immunization. With older children and adults the Schick test should be done first and only the positive reactors selected for immunization.

Children bear the injections better than adults, but immunization may be safely practised at any age; the best time, however, is during early childhood from six months to six years of age.

Reactions.—Adults and older children may develop local reactions of slight edema, erythema, and tenderness regarded as anaphylactic reactions to the dissolved proteins of the diphtheria bacilli in the toxin. Children under four years commonly do not show any reaction. *Persons who have shown a combined true and pseudo-Schick reaction are especially likely to develop a local reaction*, the latter indicating a hypersensitiveness to the proteins in the toxin. Occasionally a local reaction is accompanied by slight fever and malaise. I have never observed serum sickness, probably because the amount of concentrated antitoxin actually injected is too small to elicit this general reaction. According to Park in children of school age about 10 per cent. develop fairly sore arms and temperatures of from 99° to 103° F. About 5 per cent. feel miserable enough to stay at home from school for one day, and a very few for two days. In adults the reactions are apt to be more frequent and severe, but not quite as marked as follows injections of typhoid-paratyphoid vaccine. In infants the reactions are practically negligible.

Probably some degree of anaphylactic sensitization to horse-serum proteins is engendered by the injection of T-A, but the degree of sensitization is very slight and does not constitute a contraindication to the administration of horse antiserum (like tetanus antitoxin, antimeningococcus serum, etc.) at a later time in life.

¹ Jour. Amer. Med. Assoc., 1922, 79, 1584.

The Development of Immunity.—After each injection a portion of the toxin becomes dissociated from the antitoxin and serves to stimulate the body cells with the production of antitoxin, but the process is slow and generally three or six months are required after the last dose for the production of sufficient antitoxin to render the patient Schick negative.

According to Zingher one injection of T-A will immunize about 60 per cent. of Schick positive reactors; two injections immunizes about 80 per cent., and three injections about 90 to 95 per cent. By reinjecting those who still give a well-marked positive Schick reaction at the end of three months, with two or three more doses of T-A, an active immunity may be developed in almost all susceptible persons. If the Schick test is only faintly positive one additional injection of T-A will probably suffice.

Test for Immunity.—It is possible to actually measure the amount of antitoxin produced in the blood as described in Chapter XIII; recently Kellog¹ has described a new test for this purpose which is described in the same chapter. For practical purposes, however, the Schick test is commonly employed and when *properly conducted* and found to yield a *negative reaction*, is a safe index of a sufficient degree of immunity to diphtheria. As stated above, the test should be conducted three to six months after the last injection of T-A.

Duration of Immunity.—According to Park and Zingher, who have had an extensive experience in New York, the immunity after three doses of T-A, prepared as described above, apparently lasts for six years and the indications are that it may persist for a lifetime. A child successfully immunized at four years of age may expect protection at least until twelve to fifteen years of age, at which time natural immunity will probably have developed to such degree as to afford protection for the balance of life. Schroeder² found in one series of children that the immunity persisted for five years, and in a second group of 570 children immunity had persisted for at least two years in 90 to 95 per cent. Meyer³ has observed that immunity has persisted in 94.4 per cent. of a group of children for at least forty-four months. It is entirely likely that retesting (Schick) will show that the majority of these children are still immune and that they will continue to yield negative reactions.

Dangers and Contraindications.—When the T-A mixtures are properly prepared extensive experience has shown that the method is without danger. Zingher reports that many thousands of injections have been given with the toxin-antitoxin preparation of the New York Research Laboratory of the Bureau of Health without a single untoward result. Deplorable accidents have occurred due to the injection of mixtures too highly toxic for the guinea-pig and these emphasize the necessity for extreme care in preparing and testing the mixtures. According to Zingher mixtures properly prepared always remain safe, that is, do not acquire increased toxicity and remain effective for immunization for at least six months.

Von Behring advises against the immunization of atrophic infants and individuals suffering with acute active tuberculosis. The fear expressed by some that immunization is contraindicated in those persons harboring diphtheria bacilli or in the presence of an epidemic, by reason of producing a negative phase of temporary depression of resistance, has been finally dissipated by practical experience. Not one of numerous carriers injected with T-A has developed diphtheria. Whether or not active immunization

¹ Jour. Amer. Med. Assoc., 1922, 78, 1782.

² Arch. Pediat., 1921, 38, 368.

³ Jour. Amer. Med. Assoc., 1922, 78, 716.

with T-A aids in getting rid of the bacilli is still an open question, but very probably does not have this effect.

Practical Value.—Abroad von Behring and his assistants¹ and in this country Park and Zingher² have employed this method of immunization against diphtheria on a broad scale and have amply demonstrated the safety and practical value of the procedure. Blum,³ Meyer,⁴ Lilly,⁵ White,⁶ Mulsow,⁷ and others have reported favorably. My own experience has likewise been most favorable in that I have observed no accidents or untoward results aside from a large local reaction in an occasional adult. In about 88 per cent. of Schick positive children three injections have engendered sufficient antitoxin in my experience to yield negative Schick reactions in tests conducted about four months after the last dose.

Park and Zingher have observed that the immunity produced by three injections of T-A prepared by them has persisted for at least seven years, and the indications are that it may persist in the majority of individuals for life. This opinion is based upon the results of Schick tests, but so far there is a very large amount of evidence to show that a negative Schick reaction *properly conducted* is a sure sign of sufficient antitoxin immunity to protect against diphtheria.

The results, therefore, are better than obtained in typhoid immunization and approach in perfection the immunity engendered against smallpox by vaccination. Park has recently reported the following results in New York City:

(a) Among 57,000 originally Schick-negative children, 5 cases of diphtheria were reported. It is possible that some of these were not true clinical diphtheria, but cases of tonsillitis showing carrier bacilli in the cultures.

(b) Among 33,000 Schick-positive children who received one to three injections of toxin-antitoxin, 9 cases of diphtheria were reported.

(c) Among a total of 90,000 Schick-negative or immunized children, 14 cases of diphtheria were reported, whereas among a total of 90,000 untreated control children, 56 cases of diphtheria were reported. That is, *about four times as many cases of diphtheria developed among the unvaccinated children.*

Diphtheria still remains an active and widely prevalent disease throughout the world even though the mortality has been reduced from 70 to 75 per cent. to 10 per cent. since the advent of antitoxin in 1894. For the United States it is calculated that the yearly incidence of the disease is from 150,000 to 200,000 cases per year and the deaths 20,000 to 22,000 per year. Despite vigorous public health measures and the prophylactic use of antitoxin, the disease demands greater efforts toward its eradication, and for this purpose the wide-spread use of active immunization with T-A mixtures, is proving most valuable.

Recommendations.—1. *In the presence of exposure to diphtheria when rapid protection is required antitoxin should be injected;* the immunity engendered by T-A mixtures develops too slowly for this purpose. It is to be remembered, however, that *antitoxin protects for only a few weeks.*

¹ See Semaine médicale, 1913, xxxiii, No. 18; Berl. klin. Wochenschr., 1914, lix, 917; *ibid.*, 1914, lix, 917; Therap. Monatschr., 1913, xxvii, No. 11; Deutsch. med. Wchnschr., 1913, xxx, 460; *ibid.*, 1913, xxix, 977; *ibid.*, 1913, xxxix, 1977; *ibid.*, 1913, xxxix, 2500; *ibid.*, 1914, xl, 13; *ibid.*, 1914, xl, 582.

² Jour. Amer. Med. Assoc., 1914, 63, 859; *ibid.*, 1915, 65, 2216.

³ Amer. Jour. Dis. Child., 1920, 20, 22.

⁴ Jour. Amer. Med. Assoc., 1922, 78, 716.

⁵ Boston Med. and Surg. Jour., 1920, clxxxii, 110.

⁶ *Ibid.*, 1921, 184, 246.

⁷ Jour. Amer. Med. Assoc., 1921, 77, 1254.

2. Physicians, nurses, and others especially likely to be exposed to diphtheria should have the Schick test conducted and if positive should be immunized with T-A mixtures. This is especially advisable for nurses who may thereby avoid the disagreeable effects of one or more injections of antitoxin during their training and in subsequent practice.

3. Since diphtheria is pre-eminently a disease of children, it may be said that the Schick test and T-A immunization have proved so reliable when properly administered that all children known to be susceptible to diphtheria should be immunized. This is particularly true of children in institutions, as hospitals and asylums, where close contact increases the incidence, but even in private practice physicians should acquaint parents with the advantages of these procedures and advise their use.

Zingher¹ has demonstrated in the schools of New York City that it is possible to conduct the Schick test and apply T-A immunization on a large scale, and it is very much hoped that other cities and states adopt similar procedures for the solution of the diphtheria problem.

4. The Schick test is a great aid, but may be dispensed with under certain conditions as stated by Zingher²:

(a) *Infants under six months* do not require the Schick test or active immunization, inasmuch as 80 to 90 per cent. are immune to diphtheria (presumably inherited from the mother). Furthermore, Zingher has shown that they do not produce antitoxin very well even when immunized.

(b) *All children six months to two years of age* should be immunized, as this is the most important period. The Schick test may be omitted because it is not reliable at this age, inasmuch as a child reacting negatively may lose its natural immunity and later react positively.

(c) *Children two to five years of age* may first be tested by the Schick test because after two years a negative reaction is reliable, inasmuch as it remains constant. But since the majority of children react positively the Schick test may be omitted as a routine procedure and active immunization applied to all.

(d) *Children six to fifteen years and adults* should first have a Schick test; T-A immunization is only required when the reactions are positive.

(e) Immunity to diphtheria after T-A immunization can never be assumed to have been produced; the only reliable evidence of this is actual demonstration of antitoxin in the blood and for this purpose the Schick test should be conducted three to six months after the last injection.

SERUM PROPHYLAXIS OF TETANUS

Immunity in Tetanus.—Tetanus occurs spontaneously in man, horses, cattle, sheep, and rarely in dogs and goats. Birds and chickens are highly resistant.

It is impossible to state whether or not human beings possess natural immunity analogous to the natural antitoxic immunity possessed by many individuals to diphtheria. Since infants may suffer from tetanus infections of the umbilical stump, children of all ages from vaccinal and wound tetanus, and adults of both sexes and all races from wound tetanus, it is highly probable that natural antitoxic immunity to tetanus is very uncommon or does not exist to sufficient degree to confer immunity. Studies of the blood of human beings for tetanus antitoxin have generally failed to detect its presence; Lowry³ examined the sera of 5 normal persons, with negative

¹ Jour. Amer. Med. Assoc., 1921, 77, 835.

² Jour. Lab. and Clin. Med., 1920, 6, 117; Amer. Jour. Dis. Child., 1918, 16, 83

³ Wien. klin. Wchn., 1915, 28, 1273.

results. Escape from tetanus infection is to be ascribed more to fortuitous circumstances, preventing the growth of the bacilli and production of toxins, than to the presence of natural immunity principles aside from the degree of protection afforded by phagocytosis of bacilli in the tissues. Necrotic tissue products tend to decrease the degree of phagocytosis as well as favoring the production of tetanus toxins.

The mortality of the disease is very high. Our body cells react slowly to stimulation by tetanus toxins with the production of antitoxin. For this reason the bacilli and spores may live for several weeks in wounds, the individual being protected in the meantime by horse-serum antitoxin, only to produce tetanus when the heterologous antitoxin has been eliminated. I have not been able to find data bearing upon the question of duration of immunity after recovery. It is highly probable that the immunity is of short duration and the individual subject to reinfection, but records of two attacks at different times in the same person have not been found. Both Lowry and Wintz¹ have found, however, that human beings produce antitoxin during tetanus infection.

Natural resistance to tetanus is probably almost entirely cellularphagocytic. Recovery from tetanus is probably largely by means of the neutralization of the free and to some extent of the combined toxin, by antitoxin aided by the phagocytic destruction of the bacilli in the infected tissues.

Dangerous Wounds from the Standpoint of Tetanus.—One of the greatest dangers from this terrible infection lies in the fact that while the local lesion may show no signs of disturbance, the central nervous system may suddenly manifest symptoms of poisoning. *The wounds that are likely to contain the tetanus bacillus are the following:* All wounds that may contain dirt contaminated by manure, such as that from the streets, stables, barns, and even fields; wounds made by firecrackers or toy pistols; gunshot wounds, especially those made by blank cartridges; crushing injuries, made by machinery or in other ways. The feet and hands are especially prone to be infected with tetanus germs. Crushing injuries of bones are especially dangerous and, as shown during the late war, frost bites with phylctenules, ulcerations, and gangrene produce many cases of tetanus. Stone² has recently reported that of 49 cases of tetanus, 46.6 per cent. followed injuries of the feet and legs and 24.3 per cent. followed injury of the upper extremity. Street injuries that are not deep or perforating, but grinding and lacerating, are very likely to develop tetanus infection. It has also been stated that tetanus bacilli may be harbored in an old injury, and yet cause no symptoms until some additional injury or general disturbance of the body causes the normal protection against infection to be broken down, when toxins from the bacilli may be absorbed and tetanic symptoms develop. This theory would seem to be responsible for an otherwise apparently unaccountable development of tetanus.

From what has been said it will be seen that any injuries received on the street, or those inflicted on workers about horses or cattle and in stables, are more likely to develop tetanus than are injuries received in other ways. Newborn babies may be infected through the stump of the umbilical cord. Likewise a suppurating wound, or even a fresh wound, which may be innocent at first, may become infected with the tetanus bacillus if the wound or suppurating focus is improperly cared for. Many cases of vaccinal tetanus can thus be accounted for, *i. e.*, due to negligence in the care and treatment of the wound. *It is now generally agreed that proper treatment of the original*

¹ Münch. med. Wchn., 1915, 62, 1561.

² Jour. Amer. Med. Assoc., 1922, 78, 1939.

wound, combined with the administration of tetanus antitoxin, will surely prevent the development of lockjaw.

Value of Antitoxin Prophylaxis.—In former years Fourth of July wounds claimed a heavy toll of fatalities due to tetanus. Owing to the efforts of the American Medical Association municipalities have been urged to adopt legislative measures for enforcing a saner form of celebration, and efforts have been made to educate physicians in the proper care of these wounds and to impress upon them the great prophylactic value of tetanus antitoxin. These efforts have been crowned with success, as statistics collected from all parts of the country will show. In 1903 there were in the United States 406 deaths from tetanus; in 1904, 91; in 1905, 87; in 1906, 75; in 1907, 73; in 1908, 76; in 1911, 18 cases and 10 deaths; in 1912, 7 cases with 6 deaths, and in 1913, 4 cases with 3 deaths.

Experience during the recent war has furnished ample new evidence of the great value of serum prophylaxis. Since tetanus bacilli and spores are to be found in dirt and earth, the earth-digging methods of warfare practised during the early period of this war were responsible for a high incidence of tetanus, and especially in wounds grossly contaminated with dirt and foreign bodies and in wounds involving bones. During the first years the sudden demand for antitoxin was met by the preparation of sera below the average in potency, so that disappointments in its protective value were encountered, but with this correction the evidence of army surgeons, as Tizzoni,¹ MacConkey,² Bruce,³ Bazy,⁴ Leischman,⁵ Vaillard,⁶ and others,⁷ are overwhelmingly in favor of the high prophylactic value of the serum given subcutaneously or intramuscularly.

In the American Army the prophylactic dose of serum was 1500 units by subcutaneous injection as soon as possible after injury. This was repeated ten days later and again when subsequent operation was done. Of 224,089 injured men only 36 developed tetanus or one to each 6224 wounds (Stone). This is in marked contrast to the incidence of tetanus in the Civil War when, according to Sanford,⁸ the incidence was one case of tetanus to each 487 wounds. Bazy⁹ observed 200 wounded men in the same sector in France; 100 received antitoxin and none developed tetanus, while of 100 who were not immunized, 18 cases developed.

In the British Army, Bruce¹⁰ has stated that the incidence of tetanus among the immunized never rose above 2 or 3 per thousand; before the routine use of antitoxin the incidence was from 15 to 32 per thousand.

The British statistics also show that of the wounded immunized with 500 units of antitoxin, the average period of incubation among those developing tetanus was 45.5 days with a mortality of 22.5 per cent.; among those not immunized the average incubation period was 10.9 days and the mortality was 53.3 per cent. These figures indicate therefore, that *prophylactic immunization not only lengthened the period of incubation, but greatly reduced the mortality from tetanus even when the disease developed despite the injection of antitoxin.*

¹ Gazz. d. osp. e d. clin., 1914, 35, No. 56.

² Brit. Med. Jour., 1915, 2, 849.

³ Brit. Med. Jour., 1917, 1, 118.

⁴ Lancet, 1918, 2, 523.

⁵ Lancet, 1917, 1, 131.

⁶ Bull. d. l'Acad. d. Med., 1916, 76, 167.

⁷ Amer. Jour. Med. Sci., 1919, clvii, 764.

⁸ Bull. Internat. A. M. Museum, 1918, 7, 365.

⁹ Bull. et mem. Soc. de Chir., 1916, 42, 2919.

¹⁰ Brit. Med. Jour., 1917, 1, 118.

While it is not within the province of this chapter to deal with surgical technic, the proper cleansing and care of a wound constitute so important a part of the prophylaxis of tetanus that I shall refer to this subject, quoting largely from the technic described by Ashhurst and John.¹

Prophylactic Surgical Treatment of Wounds.—1. The surrounding skin should be painted with a 3 per cent. alcoholic solution of iodine; for simple wounds this will ordinarily suffice.

2. All foreign material should be removed from the wound, and to do this properly all parts of the wound should be made accessible by wide incision, under ether, if necessary. This is especially true of a puncture wound. It should then be freed from all tags and loose shreds of tissue by means of the scissors, and the whole wound swabbed with the 3 per cent. iodine solution. The wound should next be dressed with gauze soaked in the same solution. The use of strong caustics is inadvisable, as they cause sloughing and tend to produce a good focus for the growth of tetanus bacilli. Bruce² has stated that in lacerated wounds primary excision of the tissues should be done before the wound is sutured. The cautery should not be used.

3. The wound should be dressed daily at first, being exposed and thoroughly irrigated with hydrogen dioxide solution, and then dressed with the gauze saturated with the iodine solution. As soon as healthy granulations have formed, balsam of Peru applications should be made.

4. Antitetanic powders have been prepared, made up with antiseptics, and although Robertson³ and others have found experimentally that their use has seemed to be successful in preventing the development or absorption of tetanus toxin, still it has not as yet shown that these results were not merely due to the strong antiseptic that was combined with the antitoxin powder. It might, however, be well to apply tetanus antitoxin and antitetanic powder to the open wound, *but these remedies are not to be relied upon nor accepted as substitutes for the injection of antitoxin.*

Serum Prophylaxis.—The most successful preventive treatment, and practically the only successful curative one after the disease has developed, is by means of tetanus antitoxin. As a prophylactic remedy this antitoxin exceeds in value even diphtheria antitoxin; therapeutically, however, it is far inferior to the latter, for the reason that part of the toxin produced by the tetanus bacilli soon unites with the nerve-cells of the spinal cord where it may not be neutralized. The prophylactic use of tetanus antitoxin, however, has not infrequently been unsuccessful, due probably to the fact that it was used incorrectly.

1. Antitoxin should be given as soon as possible after the wound has been inflicted, and best at the time the primary treatment is given. The antitoxin should be injected "as near the wound as possible, so as to flood the tissues in the immediate vicinity," and, if possible, it should be given intramuscularly, so that the motor nerves may absorb it rapidly. If any nerves are exposed, antitoxin should be injected into them. *The injection of 1500 units of antitoxin is generally advised as the first prophylactic dose.*

2. From the fact that tetanus antitoxin is one of the albuminous constituents of horse-serum that are foreign to the human system, in the human being the antitoxin is rapidly eliminated in from eight to ten days after the injection is administered. Knorr has found, as the result of animal experiments, that by the sixth day only one-third, and by the twelfth day only

¹ Amer. Jour. Med. Sci., 1913, cxlvi, No. 1.

² Jour. Hyg., 1920, 19, 1.

³ Amer. Jour. Med. Sci., 1916, 151, 668.

one-fiftieth, of the optimum quantity remained in the blood. *Hence it is important, if antitoxin is to prove useful, that it should be present in the system for two or three weeks after receipt of the injury, especially as it cannot be determined when the tetanus bacillus first gained access to the wound. There should be a second intramuscular injection of 1000 units of antitoxin about the end of the first week, and perhaps a similar dose at the end of the third week in severe cases.* While certain individuals may develop serum sickness, no dangerous symptoms have been observed to result from the use of tetanus antitoxin.

3. If the surgeon is first consulted several days after the injury has been inflicted, and there are reasons for suspecting that tetanus infection has occurred, the wound should be opened and dressed as previously described, and, in addition to the intramuscular injection of 1500 units of antitoxin in the neighborhood of the wound, it will be good practice to inject an additional 5000 or 10,000 units *intravenously*. It requires at least twenty-four hours for the antitoxin to be absorbed from the tissues, and immediate neutralization of any toxin present in the blood may mean a great deal from the standpoint of prognosis if tetanus should develop.

SERUM PROPHYLAXIS OF BACILLARY DYSENTERY

Antidysentery sera are prepared by the immunization of horses with the Shiga dysentery bacillus and its toxins; the serum is largely in the nature of an antitoxin. Horses may also be immunized against bacilli of the Flexner group; the serum is largely antibacterial. A polyvalent serum may be prepared by mixing these, or horses may be immunized simultaneously with Shiga and Flexner bacilli and toxins. Antiserum for the Shiga toxins will not protect against infections with the Flexner bacilli, and the reverse.

For prophylactic purposes 10 c.c. of polyvalent serum may be injected subcutaneously. The immunity only lasts for twelve to fourteen days.

Prophylactic immunization has its greatest value in institutional outbreaks of bacillary dysentery. The serum is of no value in amebic dysentery or in non-dysenteric ileocolitis of children.

SERUM PROPHYLAXIS OF GAS GANGRENE

Bacteriology of Gas Gangrene.—The bacteriology of gas gangrene was the subject of a great deal of investigation during the World War because 0.5 to 3.5 per cent. of wounded developed this dreadful complication with a mortality of 10 to 50 per cent., depending upon how early treatment was given. Until these studies were made *Bacillus aërogenes capsulatus* (*B. welchii*, *B. perfringens*) was regarded as the sole cause of this infection, but experience has shown that pure infections of war wounds with this bacillus were so rare as to be almost negligible. Other spore-bearing anaërobic bacilli were commonly present in gas gangrene, as *B. œdematis maligni* (vibrio septique) and *B. œdematis* (*B. bellonensis*), although frequently overgrown by other micro-organisms.

Immunity in Gas Gangrene.—Immunity in gas gangrene is probably similar to that in tetanus. Nothing is definitely known of natural immunity to the various micro-organisms and their toxins identified with these infections. In all probability the serum of normal human beings possesses little or no neutralizing effects upon these toxins. Phagocytosis is probably the chief means of defense and resistance to infection; phagocytosis is likewise, probably, the chief mechanism concerned in recovery and especially after the negative chemotactic substances (toxins and products of necrotic tissue) are removed by drainage. As in diphtheria and tetanus, antitoxin produc-

tion against the toxins of gas gangrene by our body cells is probably tardy and never reaches a high degree.

Preparation of Immune Sera.—Bull and Pritchett¹ have found that *B. aërogenes capsulatus* (*B. welchii*, *B. perfringens*) produces an exotoxin in fluid culture-media, and that horses immunized with sterile filtrates containing the toxin produce an antitoxin affording protection to pigeons and guinea-pigs infected with the bacillus or its soluble toxin. The part played by this bacillus in gas gangrene, however, is of minor importance; some investigators regard it as a saprophyte, and Taylor² and others doubt that the hemolytic poison produced by its growth in muscles is a true exotoxin for which an antitoxic serum can be produced. While Bull's antitoxin is undoubtedly protective and curative in experimental infections of laboratory animals, the serum has proved generally disappointing in the prevention and treatment of gas gangrene of war wounds; Gibbon³ found that it sometimes arrested infections, had no effect on others, and occasionally produced severe reactions.

Caulfield⁴ has recently described in detail a method for the immunization of horses with toxins of *B. perfringens* and other gas-producing organisms; also methods for maintaining the virulence of the cultures, preparation of the toxins, etc.

The standard adopted by the Hygienic Laboratory for *B. perfringens* antitoxin has been recently described by Ida Bengtson.⁵ The unit shall be 1 c.c. of the standard serum which is kept in cold storage. To estimate the potency of a fresh serum, the test toxin shall first be standardized by inoculating pigeons intramuscularly with 1/100 unit of standard serum mixed with varying amounts of toxin to determine the smallest amount of toxin which will overcome this amount of serum and kill the pigeon within twenty-four hours. This dose of toxin, called the "test dose," is usually somewhat greater than 10 minimal lethal doses. The test dose of toxin is then to be mixed with varying amounts of the serum to be standardized and injected into a second series of pigeons; that amount of serum which gives protection against the test dose shall be considered to contain 1/100 unit.

Antisera have also been produced in the Pasteur Institute of Paris by Weinberg and Séquin,⁶ Leclainche and Valleé,⁷ and Sacquépée⁸ by immunizing horses with dead and living cultures of *B. aërogenes capsulatus* (*B. welchii*, *B. perfringens*); this serum is designated as antitoxin serum; for *B. œdematis maligni* (*vibrio septique*), designated as antivibrio septique or anti-œdematous serum, and for *B. œdematiens* (*B. bellonensis*), designated as anti-œdematiens or antibellonensis serum. In an experimental study of these sera Nevin⁹ found that Bull's antitoxin alone was ineffective in mixed infections and that best results followed the use of a mixture of these various antisera. These results appear to be in accord with clinical opinions, namely, that the antitoxic serum for *B. welchii* alone is unsatisfactory, and that best results in the serum prophylaxis and treatment of gas gangrene may be expected with a mixture of the antisera for *B. welchii*, *B. œdematis maligni*, and *B. œdematiens* or a polyvalent serum secured by immunizing

¹ Jour. Exper. Med., 1917, 26, 119.

² Bull. Johns Hopkins Hosp., 1916, 26, 297.

³ Amer. Jour. Med. Sci., 1919, 157, 764.

⁴ Jour. Infect. Dis., 1920, 27, 151.

⁵ Hygienic Lab. Bull., No. 122.

⁶ Proc. Roy. Soc. Med., 1916, 9, 119-144.

⁷ Bull. et mém. Soc. de chir. de Paris, 1916, 42, 1804-46.

⁸ Presse méd., 1918, 26, 197-199.

⁹ Jour. Infect. Dis., 1919, 25, 178-188.

horses with the three micro-organisms and their products. For example, Duval and Vaucher¹ have reported favorably upon the prophylactic value of such sera, the mortality rate being reduced from 16 to 3.5 per cent.; of 77 cases of gangrene treated with serum, 16, or 20.7 per cent., died. Marquis,² in the treatment of 10 cases with serum, lost 2, a mortality of 20 per cent.; Rouvillois, Guilleme, Louis, Pedeprade, and Thibierge³ treated 25 cases, 3 of whom were moribund, with a mortality of 24 per cent. Mairesse and Regnier⁴ had 30 cases, or 10 per cent., of 297 wounded men showing gas bacillus infection, develop gangrene after the prophylactic administration of antiperfringens, antivibrio septique, or antiœdematiens sera, depending upon the bacteriology; of 30 cases receiving curative injections the mortality was 16 per cent.

Van Beuren⁵ states that the prophylactic use of antigas serum in the British and American armies yielded favorable and encouraging results, and both the Third and Fifth Interallied Surgical Congresses for the Study of War Wounds reported that antiperfringens serum seemed to have given favorable results as a prophylactic, and that antivibrio septique and antiœdematiens sera yielded appreciable results, both from a prophylactic and curative standpoint. All investigators are agreed, however, that serotherapy is but an adjuvant to surgery, and that surgical treatment "is still indicated and in no way modified." In general the lowest mortality from gas gangrene treated by operation alone is about 25 per cent., and by operation plus serotherapy about 19 per cent.

Serum Prophylaxis.—In the treatment of gas gangrene best results appear to have followed: (1) A prophylactic dose of polyvalent antigas gangrene serum and tetanus antitoxin; (2) earliest possible surgical treatment and bacteriologic examination of the wound; (3) the administration of sera, either single or polyvalent, according as there are one or more gas bacilli in the wound.

Intravenous injections of polyvalent serum in combination with deep muscular injections proximal to but in the vicinity of the wound are to be preferred; the total amount injected for prophylaxis should be from 30 to 100 c.c., half intravenously and half intramuscularly. The serum treatment of gas gangrene is described in Chapter XL.

SERUM PROPHYLAXIS OF MENINGITIS

Prophylactic Immunization in Meningococcus Meningitis.—In Chapter XXXV mention has been made of the probable value of *active immunization* against epidemic meningitis by the subcutaneous injections of three doses of a polyvalent meningococcus vaccine at intervals of a week. Sophian and others have shown experimentally that opsonins, bacteriolysins, agglutinins, and other antibodies are produced, and while meningococcus meningitis is ordinarily but mildly infectious (about 5 per cent. of secondary cases in homes), the method is practically devoid of danger and worthy of trial, especially for physicians, nurses, and members of a household who are exposed to the infection over a period of many weeks.

Passive immunization by means of the subcutaneous injection of anti-meningitic serum was advised by Jochmann in 1906, but has not come into general use. The immunity resulting from the injection of from 10 to 20 c.c.

¹ Bull. et mém. Soc. de chir. de Paris, 1918, 44, 1535.

² Bull. et mém. Soc. de chir. de Paris, 1918, 44, 1522.

³ Bull. et mém. Soc. de chir. de Paris, 1918, 44, 1226.

⁴ Presse méd., 1918, 26, 461, 462.

⁵ Jour. Amer. Med. Assoc., 1919, 63, 239-242.

of serum is only temporary, and probably lasts about a month. Another drawback is the resulting serum sensitization, which renders subsequent injections of serum more likely to be followed by serum sickness. In the presence of an active epidemic, such as that which occurred in Texas during 1912, immediate passive immunization of physicians, nurses, and attendants by the subcutaneous injection of 15 c.c. of serum, followed by active immunization with three doses of vaccine (500, 1000, and 1000 millions) at intervals of a week, may be advisable. While there are no available statistics to prove the value of this procedure, it is, at least, a rational one, and since there is danger of contracting the disease, especially after prolonged contact, physicians should practice immunization during epidemics of this dreaded disease. In mixed passive and active immunization the serum probably affords immediate protection, and tides over any temporary negative phase or period of lowered resistance following the injections of vaccine.

SERUM PROPHYLAXIS OF MEASLES

Owing to the high mortality in measles of children between the ages of six months and five years attempts have been made to immunize against the disease and to reduce its severity, at least over this period of greatest susceptibility. In Chapter XXXV mention was made of the work of Hermann, who employed active immunization by means of injections of nasal secretions taken from children with measles. This method, however, may excite a severe attack, and more attention has been given the possibility of developing a successful form of passive immunization by the injection of blood or serum taken from measles convalescent patients.

Degkwitz¹ has injected 3 c.c. or more of serum taken from cases of measles between the seventh and fifteenth days after the disappearance of fever, into 172 children exposed to the disease, and states that all of these escaped the disease. Richardson and Connor² injected 7 to 25 c.c. of serum and reported that 6 were definitely exposed to measles and escaped infection; one control case developed measles. Eight others were partially exposed and escaped infection. In 3 others serum and nasal virus were injected simultaneously; in 2 there was no reaction, while the third developed a mild atypical rash. Maggiore³ injected susceptible children with 2 c.c. of serum upon entrance into the hospital and double this amount the following day. Kutter⁴ reports that of 145 susceptible children injected after the method of Degkwitz success was complete in 107 instances. Rietschel⁵ also reports favorably upon this method of immunization, but draws attention to the difficulty of securing supplies of serum from young children; the serum of adults who had had measles in childhood was found less effective. v. Torday⁶ inoculated 261 children with amounts of serum varying from 3.5 to 22 c.c.; all had been exposed to measles and only 15 developed the disease. The short duration of the immunity is indicated by the fact that 3 of the children developed measles from seventy-two to seventy-five days later. Nicolle and Conseil⁷ also report successful immunization by this method. McNeal⁸ inoculated 16 children exposed to measles with 5 c.c. of serum

¹ Ztschr. f. Kinderh., 1920, 25, 134; *ibid.*, 1920, 27, 171.

² Jour. Amer. Med. Assoc., 1919, 72, 1045.

³ *Pediatrica*, Naples, 1921, 29, 873.

⁴ Ztschr. f. Kinderh., 1921, 30, 90.

⁵ Ztschr. f. Kinderh., 1921, 29, 127.

⁶ Ztschr. f. Kinderh., 1921, 29, 148.

⁷ Bull. d. l. Soc. méd. d. hôp., 1918, 42, 336.

⁸ Jour. Amer. Med. Assoc., 1922, 78, 340.

intramuscularly; of these 4 developed the disease in a mild form. Hirasahi and Okamoto¹ inoculated 44 children with blood taken from cases between the time of appearance of Koplik's spots and the height of the eruption. The initial dose was small, being about 0.0001 c.c. of blood. A second injection was given about three weeks later. These investigators were inclined to regard this a form of active immunization with virus in the blood and regard the method as tending to increase resistance without conferring an absolute immunity.

Collection and Administration of Serum.—Blood should be removed aseptically from children with measles from the seventh to fifteenth day after the disappearance of fever and preferably from the seventh to ninth days. The children should be free of syphilis and tuberculosis. The serum is collected, cultured for sterility, and preserved with 0.5 per cent. phenol. The dose for immunization of children should be 5 to 10 c.c. by subcutaneous or intramuscular injection. The injections should be made as soon as possible after exposure; injections made late in the period of incubation may not prove prophylactic, although apparently mitigate the severity of the attack. Reactions have not been reported, and since the serum is homologous, there is no danger of anaphylaxis.

The duration of the immunity is apparently not over sixty to seventy days.

As stated by McNeal, the method recommends itself for the prevention of measles between the ages of five months and six years, and especially in institutions in which large numbers of frail children are intimately associated.

Naturally these results indicate that similar attempts in passive immunization against scarlet fever may prove worthy of investigation.

SERUM PROPHYLAXIS OF MUMPS

Immunity in Mumps.—Mumps or epidemic parotitis occurs only in man. The etiology is unknown.

Infants possess some degree of natural immunity, inasmuch as the disease is uncommon under one year of age. After this time children are highly susceptible and in institutions as many as 90 per cent. may become infected. Adults enjoy a certain degree of immunity, but this is not absolute; in men the testicles may become involved and in women the ovaries and mammary glands.

Both sexes and all races are susceptible, but boys are probably more susceptible than girls.

One attack generally confers a lasting immunity; second attacks, however, may occur.

This type of parotitis is probably different from "postoperative parotitis" which sometimes follows injuries and operations upon the pelvic and abdominal organs.

Serum Prophylaxis.—Hess² has employed intramuscular injections of whole blood for prophylactic immunization. The dose was 6 to 8 c.c. by intramuscular injection. The donors were selected from children just recovering from mumps and those who had had the disease from ten days to one or two years previously. Of 17 children inoculated none contracted the disease although intimately exposed, and Hess believes that these inoculations conferred some degree of immunity and aided in checking an epidemic.

¹ Japan Med. World, 1921, 1, 10.

² Amer. Jour. Dis. Children, 1915, 10, 99.

SERUM PROPHYLAXIS OF SYPHILIS

The immunity of syphilis and active immunization have been discussed in Chapter XXXV.

Neisser and Bruck¹ and their associates have injected horses, sheep, and monkeys with living and dead syphilitic virus, organ extracts, and the blood of syphilitic human beings and monkeys, without being able to produce immune sera possessing the slightest effect either *in vitro* or *in vivo* upon active virus. They cite a large number of similar attempts for some of which success was claimed, but all of their experiments in addition to those of Finger and Landsteiner² and others yielded negative results.

As stated in the discussion of immunity in syphilis in Chapter XXXV, antibodies may be found in the blood of syphilitic human beings, and Eberson³ believes that spirocheticidal substances may be present in sufficient amounts to prevent syphilis in rabbits when human syphilitic serum is injected, but the amounts are too small in both human beings and the experimentally infected rabbit and monkey, to be of value in the serum prophylaxis of human syphilis. As mentioned in Chapter XXXV, animals injected with dead pallida vaccines do not produce more than very small amounts of antibodies which renders impossible the immunization of large animals, as the horse, for the preparation of immune sera; furthermore, horses are not susceptible to infection with living pallida, and at the present time it appears that nothing less than an actual syphilitic lesion with living spirochetes is required for antibody production.

SERUM PROPHYLAXIS IN DISEASES OF THE LOWER ANIMALS

The Serum Prophylaxis of Hog Cholera.—While the cause of hog cholera has not as yet been discovered, it is a well-known fact that the virus is present in the blood of infected animals, and it is possible, by immunizing healthy hogs with gradually increasing doses of infected blood, to prepare a potent immune serum that will prove of value in the prophylaxis and cure of hog cholera. The nature of this serum is unknown. It possesses many of the features of an antitoxin, and for the present may be classed with these.

Methods for the preparation of antihog cholera serum have been described in detail by Graham and Hummelberger,⁴ Haslam,⁵ and others.

Production and Standardization of Hog Cholera Serum.—Healthy hogs weighing about 100 pounds are selected, and injected subcutaneously with 40 c.c. of hog cholera serum per hundred pounds of weight. Two or three days following this protecting dose they are injected intravenously with 3 or 4 c.c. of sterile, defibrinated blood, obtained from an animal suffering from the disease; or the animals may be exposed in pens known to be infected. If the animals live for one month without showing evidences of toxemia, they receive another injection of 5 c.c. of infected blood (virus). Two or three weeks later they receive another injection of from 15 to 20 c.c. of infected blood; in from fifteen to twenty-one days after this inoculation they receive a final injection of from 4 to 5 c.c. of virus per pound of body weight. Animals tolerating the last injection are said to be hyperimmune, and are bled in ten days. In hyperimmunizing the animal some prefer to inject the virus intraperitoneally instead of intravenously. If this method is adopted, about double the dose of infected blood (virus) is required. About 5 per cent. of animals succumb during the period of immunization.

The immunized hogs are bled aseptically from the tail by snipping off the tip, and 5 c.c. of blood per pound of weight is collected in sterile vessels. Each animal is bled once a week until three or four bleedings have been made.

¹ Beitr. z. Path. u. Therapie d. Syph., 1911.

² Centralb. f. Bakteriöl., Ref., 1906, 38, 107.

³ Arch. Dermat. and Syph., 1921, 4, 490.

⁴ Jour. Infect. Dis., 1916, 18, 118.

⁵ Jour. Immunology, 1921, 6, 263.

In about one week after the last bleeding they are hyperimmunized again by giving them 4 or 5 c.c. of infected blood per pound of body weight, and additional bleedings are made so long as the tail lasts.

The serums secured in the several bleedings are mixed together. The third or fourth bleeding is said to be most potent or to contain the greatest number of antibodies.

In testing and standardizing the immune serum, six hogs, weighing about 100 pounds each, are placed in an infected pen. No. 1 receives no serum and is a control; No. 2 receives 15 c.c. of serum; No. 3 receives 20 c.c.; No. 4 receives 30 c.c.; No. 5 receives 35 c.c.; and No. 6 receives 40 c.c. of immune serum. The animals are allowed to remain in the pens until the control succumbs and the protecting dose of serum has been determined. In this manner we can determine just about the amount of serum required to protect 100 pounds of hog. The Pennsylvania Live Stock Sanitary Board has found that it does not require quite 40 c.c. of serum, but this amount is recommended for safety, and because the weight may not be judged accurately.

More recently the following method has been adopted: Seven hogs susceptible to hog cholera and weighing not less than 45 or more than 90 pounds each, are selected. Each animal is injected with 2 c.c. of the virus blood. Five of the animals are now injected with one-half of the field dose of serum which is usually about 20 c.c.

The serum test is satisfactory when both of the control pigs become sick within four to seven days after the test has started and succumb; all of the serum treated pigs must remain well throughout the test, or not more than one of these become sick subsequent to the fourth day, but fully recover before the expiration of three or four weeks, when the test is finished.

Eberson¹ and Duval and Couret² have described methods for concentrating the immune serum; whole serum, however, is generally administered.

Hog-cholera serum is used in both the prophylaxis and the therapeutic management of this disease. For *prophylactic purposes* for each 100 pounds of hog 40 c.c. of serum are injected. These injections are usually given subcutaneously and occasionally intramuscularly. In some instances active and passive immunization is practised by the simultaneous injection of 2 c.c. of virus, together with 40 c.c. of immune serum per 100 pounds of weight. Owing to the danger of spreading the disease, this method is not generally employed.

The results of prophylactic immunization are excellent, and the method is valuable in checking epidemics. Immunity is said to last for two to four months after vaccination.

Prophylaxis of Calf Cholera.—Serum has been prepared by immunizing horses with strains of colon, paracolon, and other bacilli belonging to these groups, isolated from calves dying of calf cholera. The immune serum should agglutinate the micro-organisms used in its production in dilutions of 1 : 2000 to 1 : 500.

This serum has proved of value in the prevention of calf cholera, and may be of benefit in the treatment, providing that it is prepared with the same strain or strains of micro-organisms responsible for the infection.

The amount of serum ordinarily administered is 15 to 30 c.c. by subcutaneous injection. In infected herds it is recommended that this amount of serum be injected into calves within forty-eight hours of birth. The immunity lasts for only a few weeks and subsequent injections may be required.

Serum Prophylaxis of Infectious Abortion.—A serum for the prophylaxis of infectious abortion of mares is prepared by the immunization of horses with the bacillus of equine abortion and streptococci; Good and Smith³ have

¹ Jour. Infect. Dis., 1915, 17, 339.

² Jour. Med. Research, 1921, 42, 503.

³ Jour. Infect. Dis., 1916, 18, 397.

found such sera to contain antibodies, but the practical use of the serum is still in the experimental stage. For the immunization of mares 40 to 50 c.c. serum may be injected intravenously or subcutaneously; the immunity is probably only of a few weeks' duration.

A similar serum is available for the prophylaxis of infectious abortion of cows; it is administered in the same manner and appears to confer some degree of immunity of a few weeks' duration.

Serum Prophylaxis of Anthrax.—Serum prophylaxis has not proved very effective and active immunization with vaccines or the simultaneous injection of immune serum and vaccine are generally employed. The dose of serum alone is 20 to 50 c.c. by subcutaneous injection; the resulting immunity is untrustworthy and of a week or two in duration.

Serum Prophylaxis of Botulinus.—Graham and Schwarze¹ have prepared an antitoxin for the prophylaxis of botulism in cattle which yielded encouraging results. Since *Bacillus botulinus* produces a very potent soluble toxin it would appear possible to prepare antitoxin, and that such serum may prove of value in the treatment of botulism of human beings.

Serum Prophylaxis of Rinderpest.—The cause of rinderpest, or cattle plague, so fatal among European and African cattle, is still unknown. The virus is present in the blood and bile and is filtrable through Berkefeld and Chamberland filters. The disease is characterized by inflammation of the intestinal mucous membrane. One attack usually confers a lasting immunity.

Kolle and Turner have prepared an immune serum by injecting healthy oxen subcutaneously with gradually increasing amounts of blood and bile from diseased cattle. The subcutaneous injection of 100 to 200 c.c. of immune serum is said to confer a passive immunity of several weeks' duration.

Simultaneous injections of small amounts of virus, blood, and immune serum leads to a combined active and passive immunization of longer duration and more effectiveness.

¹ Jour. Bacteriology, 1921, 6, 83.

CHAPTER XXXIX

THE PRINCIPLES OF NON-SPECIFIC PROTEIN THERAPY

Non-specific Biologic Therapy.—Within recent years considerable attention has been given the subject of “protein therapy” or the use of various non-specific agents in the treatment of disease for their therapeutic power in altering the reactivity of the whole body with general tissue stimulation and activation, rather than influencing directly the cause of the disease as in specific therapy.

In Chapter XXXIV brief mention was made of the non-specific activities of bacterial vaccines, that is, of clinical observations showing that they may exert beneficial and curative effects in diseases to which the bacteria incorporated in the vaccine have no etiologic relationship. Renaud¹ in 1911 had noted these effects with typhoid vaccine employed in the treatment of various non-typhoidal infections. In 1914 Kraus and Mazza² reported that the intravenous injection of colon vaccine produced practically the same therapeutic results in typhoid fever as the injection of typhoid vaccine, and Ichikawa³ observed equally good results in paratyphoid fever following the intravenous injection of typhoid vaccine.

From this non-specific or heterovaccine therapy it was logical to attempt the intravenous injection of other substances to elicit the shock reaction; for example, protein split products, of bacterial and non-bacterial origin, milk, sera, colloidal metals, hypertonic saline solution, water, etc. Jobling, Petersen and Eggstein⁴ were among the first to study the mechanism of the reaction following the injection of these split proteins, and found that a part of the therapeutic results must be due to the mobilization of enzymes. At the same time Lüdke⁵ reported upon the treatment of typhoid fever with the intravenous injection of albumoses, and Miller and Lusk⁶ upon the treatment of arthritis with the intravenous injection of typhoid vaccine and other foreign proteins.

Immune sera have also been administered for their non-specific effects in the treatment of disease as diphtheria antitoxin for streptococcus infections, tuberculosis, pneumonia and pleurisy, typhoid fever, dysentery, iritis, arthritis, etc. Equally good results have been observed with normal horse-serum and the effects are probably non-specific and due to serum constituents entirely apart from the antitoxin or other antibodies.

Since these pioneer investigations were made the subject of “protein therapy” has attracted considerable attention with the employment of many different substances and the accumulation of a large literature.⁷ These non-specific agents have been used solely for the treatment of disease; to the best of my knowledge none have been employed for prophylactic therapy,

¹ Presse méd., 1911, 19, 665.

² Deutsch. med. Wchn., 1914, xl, 1556.

³ Ztschr. f. Immunitätsf., 1914-15, 23, 32.

⁴ Ztschr. f. Immunitätsf., 1915-15, 24, 219; Jour. Exper. Med., 1915, 21, 239; *ibid.*, 1915, 22, 141 and 401.

⁵ Münch. med. Wchn., 1915, lxii, 321.

⁶ Jour. Amer. Med. Assoc., 1916, 66, 1756.

⁷ See Protein Therapy and Non-specific Resistance by Petersen, The MacMillan Company, New York, 1922.

although the immediate non-specific reaction following the administration of some probably aids in raising resistance to infection for a brief time.

Non-specific Agents.—The non-specific agents mostly employed for the treatment of disease have been typhoid and colon vaccines; normal and immune horse sera, cow's milk, and peptone. These, however, by no means include all. The agents of autoserum therapy, as human blood, serum and plasma, pleural exudates, leukocytic and tissue extracts, the products of sterile abscesses, altered serum proteins, etc., are included, briefly summarized as follows:

1. *Vaccines*: Stock vaccines of typhoid, colon, pyocyanus bacilli, etc. Autogenous vaccines.

2. *Bacterial extracts and yeasts*.

3. *Heterologous sera*: Normal and immune horse-serum; chicken-serum, etc.

4. *Homologous sera and blood*: Normal and convalescent human blood and serum; plasma.

5. *Homologous exudates, transudates, and secretions*: Human blister serum and pleural fluid; cerebrospinal fluid; human milk; pus; products of tissue excitation and destruction as produced by the cautery, rebofacients, turpentine, hypertonic saline solution, Roentgen rays, radium, etc.

6. *Foreign proteins and protein-split products*: Milk; casein; egg albumen; gelatin; nucleoproteins; proteoses; deuto-albumose; peptone; histamin, etc.

7. *Tissue extracts and enzymes*: Extracts of leukocytes; tumor autolysates; trypsin.

8. *Altered homologous blood proteins*: Products of hemolysis caused by the intravenous injection of water and hypotonic saline solution; altered proteins due to the injection of formalin, colloidal metals, etc.

That human blood and serum, blister fluid, pleural and other exudates and pus, the products of tissue necrosis as produced in abscesses, by the cautery, etc., should be included as "foreign proteins" for the human being appears unlikely and strange, yet their injection is capable of eliciting a response very similar to the reaction excited by the administration of truly foreign proteins, as bacterial vaccines, horse-serum, and cow's milk. Petersen believes that a part at least of the therapeutic effects following the injection of turpentine, hypertonic saline, and other substances producing "fixation abscesses," the application of rubefacients with vesiculation, the seton, cautery, etc., are due to the production and absorption of altered homologous proteins capable of eliciting in mild degree a non-specific reaction analogous to that produced by the administration of foreign proteins.

The mere withdrawal of human blood into citrate solution or the separation of serum by spontaneous coagulation or defibrination apparently results in profound changes tending to increase their toxicity and especially if administered at once. Undoubtedly withdrawal of the blood of an individual and reinjection of his serum (autoserum therapy) is sometimes beneficial in the treatment of some diseases, and the mild reaction that may be elicited appears to be quite similar to that produced by the injection of a foreign protein. In other words, our own body proteins, as our plasma, whole blood, transudates, and exudates, by very slight manipulation may be changed sufficiently to act as non-specific stimulants upon reinjection. Likewise the administration of dilute formalin solution and some of the colloidal metals appear to owe their effects to the production of sufficiently new or "foreign" products in the circulating blood to elicit non-specific reactions of the kind designated as "shock" or "foreign protein" reactions.

Probably the effects of the intravenous administration of water and hypotonic saline solution are due in part to the products of hemolysis acting as "foreign" agents.

Probably the most commonly employed non-specific agents are typhoid and colon vaccines, cow's milk, horse-serum and various split proteins, as proteoses, deuterio-albumose, and Witte's peptone. In Chapter XL will be given the doses and administration of these and other non-specific agents in the treatment of disease. Here it may be stated, however, that the reactions elicited by these agents vary considerably in degree and that the method of their preparation apparently influences their effects. For example, a vaccine of the colon bacillus generally elicits a severer reaction than typhoid vaccine and the effects of both vary according to the culture employed, method of sterilization, etc.

Leukocytic Extracts in the Treatment of Disease.—In Chapter XIX the nature of these was discussed. It has been shown experimentally by Hiss and Zinsser,¹ Petterson,² Opie³ and Zinsser, McCoy and Chapin⁴ that these extracts possess some degree of prophylactic and curative values in experimental bacterial injections of the lower animals; Youland,⁵ however, was not able to demonstrate any marked or constant degree of antibacterial activity. Hiss and Zinsser, Lambert, Lloyd and Lucas, and others have employed these extracts in the treatment of staphylococcus, streptococcus, pneumococcus, and other infections of man, to which reference will be made in the succeeding chapter.

The nature of the antibacterial substances in leukocytic extracts is not definitely known. It would appear, however, that bactericidins (leukins) may be present; likewise various enzymes. These substances are not specific for any one particular micro-organism, and their influence, therefore, is non-specific and properly included in the category of non-specific agents. Furthermore, since leukocytic extracts are generally prepared of the leukocytes of the horse or rabbit, they constitute foreign protein substances, and it is now believed that their therapeutic effects are largely dependent upon the production of the non-specific protein reaction.

The Non-specific General Reaction.—The general reaction following the intravenous injection of vaccines, sera, protein split products, etc., and the intramuscular injection of these substances in larger doses as well as milk and other agents, usually elicits a general reaction which varies greatly in severity according to the substance administered, its dosage and route of administration, the physical condition of the patient, the disease, temperature, and other individual factors. This great variation in effects, the danger of eliciting too much reaction and the lack of standardized products and dosage, greatly complicates non-specific therapy and, indeed, may render it hazardous until experience has been gained. Probably most experience has been gained with the intravenous injection of 25,000,000 to 50,000,000 of typhoid and colon vaccines and the intramuscular injection of cow's milk.

The intravenous injection of typhoid vaccine is generally followed in about half an hour (six to eight hours with colon vaccine) by chilly sensations and trembling or a frank *chill*. This lasts about twenty minutes to an hour and is treated by hot drinks, the application of hot-water bottles, etc.

¹ Jour. Med. Research, 1908, 19, 323, 399, 411, 429, 455; *ibid.*, 1909, 20, 245; *ibid.*, 1910, 22, 397; *ibid.*, 1913, 28, 385.

² Centralbl. f. Bakteriöl., 1904, 36, 71; *ibid.*, 1905, 39, 423, 613; *ibid.*, 1906, 40, 537; *ibid.*, 1906, 42, 56.

³ Jour. Exper. Med., 1905, 7, 316, 759; *ibid.*, 1906, 8, 410, 536.

⁴ Jour. Med. Research, 1911, 24, 483.

⁵ Jour. Med. Research, 1914, 31, 367.

In cases of arthritis with painful and tender joints a severe chill is to be avoided by reason of the resulting increased pain and distress. The intramuscular injection of milk is not usually followed by a chill.

As the chill subsides a *fever* develops varying from 100° to 104° F. and reaching its maximum in about three to four hours; with intramuscular injections (especially of milk) fever may not appear for six to eight hours and persist for twenty-four to forty-eight hours.

Apparently the production of fever depends more upon the individual and type of disease than upon the dosage; when the foreign agent is administered during a febrile period the temperature may not be increased, but actually show a decline.

The *pulse-rate* is generally increased by 15 to 30 beats per minute, coincident with the development of fever. In typhoid and other acute infections a pulse-rate of over 100 is regarded by Petersen as a contraindication to non-specific therapy.

Sweating generally occurs shortly after the subsidence of the chill and particularly in arthritic patients who appear to derive special temporary benefit from profuse perspiration. Sometimes there appears to be an increased production of milk in lactating women.

Frontal *headache*, *nervous irritability*, which may amount to *delirium*, *nausea and vomiting*, and *herpes* may occur and especially after the intravenous injection of vaccines in the treatment of acute infections, as typhoid fever, pneumonia, and erysipelas accompanied by considerable toxemia.

The Influence of Non-specific Agents Upon the Blood.—The most notable blood changes affect the leukocytes. As a general rule a *leukopenia* first appears, which is temporary, and followed in five to twelve hours by *leukocytosis*. The leukopenia has been ascribed to depression of the bone-marrow and accumulation of the leukocytes in the internal organs. The reactive leukocytosis is of a myeloid type—that is, due to stimulation of the bone-marrow and is largely made up of neutrophil polymorphonuclears, large mononuclears, and transitionals; the eosinophils are sometimes increased.

The degree of leukopenia and reactive leukocytosis varies with different foreign agents. Immunized animals appear to develop a higher leukocytosis than normal animals, and Gay and Claypool¹ thought that this leukocytosis was specific after the injection of typhoidin, but the phenomenon has since been shown to be non-specific. In human beings the leukocytic changes are most marked after the first injection and tend to be reduced after subsequent injections.

An *increase of erythrocytes* is commonly noted and especially in anemia; in pernicious anemia this increase is transient and uncertain. Cowie and Calhoun² have reported that the *platelets increase* in number and size after the injection of typhoid vaccine. Sometimes an increased coagulability of the blood is found due to increase of fibrinogen and thrombokinase.

The Influence of Non-specific Agents Upon Ferments and Antiferments.—Jobling, Petersen and Eggstein³ have shown that in the lower animals the injection of various non-specific agents is followed by a *mobilization of proteolytic enzymes as well as of lipases* and especially after severe reactions induced by typhoid and colon vaccines. Davis and Petersen⁴ have shown

¹ Arch. Int. Med., 1914, 14, 662.

² Arch. Int. Med., 1919, 23, 69.

³ Jour. Exper. Med., 1915, 21, 239; *ibid.*, 1915, 22, 141, 401, 568, 597.

⁴ Jour. Exper. Med., 1917, 26, 699.

similar changes in the lymph. In human beings the enzyme changes are not so marked; Petersen has summarized these as follows:

- (a) Concentration of the serum.
- (b) Practically no changes in the non-protein nitrogen of the blood.
- (c) A primary decrease of serum protease followed by a progressive increase for a period of three or four days.
- (d) An increase of serum pepsinase in cases showing clinical improvement.
- (e) No changes in the serum diastase and irregular changes in the lipases.

Jobling and Petersen have also shown that there may be an *increase of the anti-ferment (antitryptic) activity of the serum* after the injection of non-specific agents and in human beings, especially in cachexia, during acute febrile diseases, in pregnancy, serum disease, etc. These investigators have identified serum anti-ferment (anti-enzyme or antitrypsin) as consisting of finely dispersed lipoids which contain unsaturated carbon bonds in their chemical structure; they have been discussed in more detail in Chapter XIV.

The Influence of Non-specific Agents Upon Antibody Production.—In Chapter VIII mention was made of the possibility of drugs stimulating body cells to the greater production of antibody for some antigenic substance, notably pilocarpin in relation to the production of diphtheria antitoxin. Of greater importance and interest in relation to the treatment of infectious diseases is the question of the possibility of non-specific agents stimulating the production of antibodies for micro-organisms and their products.

Hektoen¹ observed that rabbits sensitized to horse-serum yielded specific antihorse precipitin when injected with some other serum; the cells sensitized to horse-serum with the production of precipitin were thereby stimulated by a non-specific agent to produce, or at least, cast off, additional amounts of specific antibody.

Similar results have been reported by Bieling² with rabbits immunized with typhoid bacilli. The subsequent injection of other non-specific substances, as colon, dysentery, and diphtheria bacilli, resulted in the production of specific typhoid agglutinin. In Chapter XX, we have noted similar observations on the non-specific production of hemolysins.

In typhoid fever, however, Baluit,³ Lüdke,⁴ v. Groer,⁵ Svestka and Marek⁶ claim that the administration of various non-specific agents did not increase typhoid antibodies, although positive results were claimed by Flachse⁷ and Parlavocchio⁸ and by Dölken⁹ in dysentery (increase of agglutinins following injections of milk). Culver¹⁰ also found that the injection of primary and secondary proteose preparations stimulate antibody production or mobilization for specific organisms in gonococcal arthritis in a manner not to be distinguished from that produced by the injection of the specific organisms themselves. Culver thought that when the injection of proteose was large enough to excite a chill, that antibody production was engendered by the consequent massage of the infected joints, but it is entirely likely that the injections of non-specific proteose stimulated the sensitized antibody producing tissues with the production or casting off of specific

¹ Jour. Infect. Dis., 1917, 21, 279.

² Ztschr. f. Immunitätsf., 1919, 28, 246.

³ Therap. Monatschr., 1915, 29, 307.

⁴ Berl. klin. Wchn., 1920, lvii, 344.

⁵ Münch. med. Wchn., 1915, lxii, 1312; Therap. Monatschr., 1916, 30, 521.

⁶ Wien. klin. Wchn., 1915, 28, 1054.

⁷ Wien. klin. Wchn., 1916, 29, 641.

⁸ Arch. f. klin. Clin., 1909, xc, 202.

⁹ Münch. med. Wchn., 1919, lxvi, 480.

¹⁰ Jour. Amer. Med. Assoc., 1917, 68, 362.

gonococcal opsonins and lysins. Uddgren¹ has observed that the intramuscular injection of milk into Wassermann negative syphilitic patients frequently acted as a provocative followed by a positive Wassermann reaction.

It is not improbable, therefore, that non-specific agents may stimulate the production or elaboration of specific antibodies *provided the antibody-producing tissues have been previously sensitized* or keyed up by the specific antigen and that a part of the good effects observed clinically after the injection of non-specific agents may be due to this antibody production. The evidence of antibody production by non-specific agents in normal rabbits or human beings not previously sensitized by vaccines or disease is very slight and probably does not occur in any constant or appreciable degree.

The Influence of Non-specific Agents Upon Metabolism.—As stated by Petersen “the nitrogen balance shows considerable variation both experimentally and clinically following the parenteral introduction of the proteins and their split products.” In patients the injection of the non-specific agents is frequently associated with an increase in the nitrogen excretion, the urine showing an increase of total nitrogen of 20 to 30 per cent. above the excretion before the injection. Petersen states that after about two days the nitrogen excretion again reaches the normal, and for a variable period after this time there exists in many patients a diminution in excretion. Diuresis may follow sometimes the injection of foreign proteins, but Uddgren² and others have not observed albuminuria or other evidences of kidney irritation.

Not infrequently patients experience a general feeling of improved well being after one or more injections of milk or some other non-specific agent and an increase of body weight; Uddgren has observed an increase of weight of some patients during a course of intramuscular injections of milk, and especially among those receiving market milk, with subsequent temperature reactions.

The Focal Reaction Following the Injection of Non-specific Agents.—In Chapter XXXIX mention was made that the injection of foreign proteins (particularly typhoid and colon vaccines intravenously and milk intramuscularly) may elicit a reaction of hyperemia, edema and pain about inflammatory foci including tuberculous lesions, analogous to the reaction about tuberculous foci excited by the subcutaneous injection of a sufficient amount of tuberculin.

This focal reaction has been commonly accepted as a specific allergic phenomenon not only in tuberculosis following the injection of tuberculin, but in other infections as well, as, for example, reactions around furuncles or other lesions after the injection of an adequate amount of specific vaccine.

As previously stated, this focal reaction is to be interpreted as both specific and non-specific. In my opinion the specific reaction is one of allergy due to allergic sensitization of the inflammatory tissues with the production of allergic cellular shock upon the introduction of the specific antigen and its union with the allergic antibody in or upon the sensitized (allergic) cells. The non-specific reaction generally requires the administration of larger amounts of agent to elicit a focal response than is required of the specific agent or antigen. The non-specific agent, however, is capable of eliciting focal reactions by means of its stimulating effect upon cellular activity (plasma-activation of Weichardt) which finds expression in increased secretory activity of gland cells, increased activity of leukocytes, etc., as

¹ Berl. klin. Wchn., 1918, lv, 354.

² Milchinjection in der Ophthalmologie, Stockholm, 1918.

well as by increasing the permeability of the lymphatics and capillaries with hyperemia and an outpouring of lymph and plasma, enzymes, etc. Doubtless the cells of inflammatory tissue about foci of infection are more susceptible of stimulation by non-specific agents than normal cells; the constitutional reaction of fever, tachycardia, etc., is probably due to the absorption of poisons from the foci, not only performed poisons derived from bacteria and disintegrated tissues, but poisonous digestion products produced by the increased enzymic activity. Schmidt¹ and Wolf-Eisner² have explained focal reactions on this dual basis; Petersen³ likewise accepts the specific as well as non-specific mechanism of the focal reaction, explaining the former on the basis of the liberation of disintegrating bacterial substance and possibly even living bacteria, and resulting in a secondary specific response on the part of the body.

Whatever may be the true explanation of the focal reaction and the relative importance of the specific and non-specific mechanisms, the fact remains that the administration of non-specific agents including not only vaccines, milk, sera, proteoses, etc., but other agents, as Roentgen rays, counterirritants, trauma, etc., may elicit focal reactions. The flare-up of tuberculous lesions after the injection of milk, proteoses, and nucleins are notable examples; likewise the exacerbations of non-tuberculous inflammatory foci in the eye, skin, joints, appendix, gall-bladder, respiratory and genito-urinary tracts, of the lancinating pains of tabes, epileptic attacks and the psychic symptoms of paresis, after the injection of these and other non-specific agents, are to be regarded as examples of non-specific focal stimulation as indicated on the reports of Schmidt and Kraus,⁴ Holler,⁵ Döllken,⁶ Josephson,⁷ v. Jauregg,⁸ and others.

The Mechanism of the Non-specific Reaction.—The effects and results of non-specific protein medication are better known and understood than the mechanism of their production. Weichardt,⁹ who has conducted a great deal of investigation in this field, believes that the essential mechanism concerned is one of cellular stimulation and that following the intravenous injection of the non-specific agent there is a universal stimulation of body cells (the *omnicellular plasma-activation theory*) to greater activity in the production of either specific antibacterial substances or merely an increase of general resistance to intoxication—either synthetic (the formation of conjugate proteins from the toxic forms) or lytic (the degradation of the toxic fragments to the amino-acids) or in some other way accelerating the elimination of the toxic substances. The theory of Döllken is similar except that he considers certain proteins and other agents as exciting a selective stimulating effect.

This stimulation does not involve any alteration in function or call into play any new method of defense; it represents rather an accumulation of the defensive agencies normally present, and for this reason non-specific therapy may be contraindicated in the terminal stages of disease when the defensive agencies are exhausted.

In addition to this cellular stimulation theory of Weichardt and Döllken,

¹ Deutsch. Arch. f. klin. Med., 1919, cxxi, 1.

² Münch. med. Wchn., 1920, lxvii, 93.

³ Arch. Int. Med., 1918, 21, 14.

⁴ Med. Klinik, 1919, 15, 503.

⁵ Med. Klinik, 1917, 13, 1038.

⁶ Münch. med. Wchn., 1919, lxvi, 480; Neurol. Centralbl., 1919, 38, 354.

⁷ Med. Record, 1920, xcvi, 607.

⁸ Psych.-neurol. Wchn., 1918-19, 20, 132, 251.

⁹ Münch. med. Wchn., 1919, lxvi, 289; *ibid.*, 1920, lxvii, 91.

which can be measured by the increased activity of the glandular parenchyma, increased motility of smooth muscle, etc., Starkenstein¹ has demonstrated an increased permeability of the endothelioma of the capillaries due probably to actual changes in the membrane of the cell followed by a decreased permeability. Due to this effect and the general cellular stimulation, enzymes, antibodies, fibrinogen, thrombokinase, and glycogen are thrown into the circulation. According to Pemberton² there seems to be some relation between the glycogen metabolism and the pathologic changes of arthritis; that as stored glycogen is exhausted improvement follows and one effect of non-specific protein therapy is to hasten the catabolism of glycogen. The alteration in the cell membrane is also indicated by augmentation of the lymph flow and by a change in the irritability of the central nervous and sympathetic nervous systems.

A combination of these two theories affords at present the most satisfactory explanation of the mechanism of non-specific protein therapy. The omniscular plasma-activation of Weichardt explains the fever due to direct or indirect stimulation of the thermoregulating mechanism, the leukocytosis due to stimulation of the bone-marrow, the increase of serum and lymph ferments, increase of antibodies providing the patient has been previously sensitized, the increase of platelets, fibrinogen, and other coagulating substances and an increase of antiferments, all of which are to be regarded as defensive agencies. The increased capillary permeability theory of Starkenstein affords an explanation of the access of these agencies to foci of disease.

The Relation of the Non-specific Reaction to Therapeutic Activity.—The treatment of disease by the administration of non-specific agents has attracted a great deal of attention during the past few years. Indeed, it would appear that this form of therapy is in danger of being overused and its importance overemphasized. One commercial firm has gone so far as to prepare different proteins, presumably of vegetable origin, for use in different diseases, apparently a specific protein for specific medication as a form of non-specific protein therapy.

Undoubtedly the effects produced by the injection of some non-specific agents are frequently beneficial, notably the intravenous injection of typhoid and colon vaccines, sera and proteoses, and the intramuscular injection of milk. The effects produced by adequate doses including general cellular stimulation, improvement of elimination and metabolism, leukocytosis, sweating, mobilization of enzymes and antibodies, are doubtless beneficial. As a general rule some reaction is required before beneficial effects are to be expected, as chilliness, moderate fever, sweating, and leukocytosis; but the degree of clinical improvement is by no means in ratio to the severity of the reactions, at least not in all diseases.

The focal reaction engendered about foci of infection may likewise prove beneficial; in my opinion, always so when of a mild character. The hyperemia, leukocytosis, and enhanced phagocytosis and mobilization of ferments resulting from the focal reactions are capable of exerting beneficial results and are desirable in the treatment of localized infections. Doubtless no small part of the success attending the treatment of tuberculosis by tuberculin and non-specific agents is due to the enhanced production of fibrous tissue following repeated mild focal reactions.

However, there is distinct danger from too severe focal reactions from the standpoint of breaking down local defensive measures and thereby

¹ Münch. med. Wchn., 1919, lxvi, 205.

² Arch. Int. Med., 1920, 25, 351.

facilitating local and general infection. This is particularly true in the treatment of tuberculosis calling for extraordinary care in the administration of tuberculin or any other agent capable of eliciting focal reactions.

The whole subject of non-specific protein therapy may be said to be still in its infancy. It is potentially a dangerous therapy by reason of the damage that may be caused by injudicious selection of the agent and error in dosage. Great caution must be exercised until more information is at hand regarding the relative merits and demerits of the different protein substances and other agents that may be used in the treatment of different diseases; likewise more information on the very important subject of dosage according to the disease and the patient as well as the route of administration to be chosen.

In the succeeding chapter will be given the treatment of different diseases by non-specific substances, as well as by vaccines and sera for specific purposes, in order to group the different forms of biologic therapy under each disease instead of handling the different subjects of vaccine, serum, and non-specific therapy in different chapters.

CHAPTER XL

VACCINES, SERA, BLOOD, AND NON-SPECIFIC PROTEINS IN THE TREATMENT OF DISEASE

IN this chapter it is proposed to present together, instead of separately, the subjects of specific vaccine and serum and non-specific protein treatment of disease. As previously discussed it is not always possible to differentiate specific from non-specific effects; indeed, it would appear that a part of the beneficial effects observed in vaccine and serum therapy are always of a general or non-specific character. The author hopes that the arrangement of this chapter will prove more serviceable than the plan pursued in previous editions of considering the subjects of serum and vaccine therapy in separate chapters.

The Administration of Vaccines, Sera, and Blood.—The technic of subcutaneous injection of bacterial vaccines is described on p. 761; of intravenous injection on p. 845. Methods for the subcutaneous, intramuscular, intraspinal, and intravenous injection of serum are described in Chapter XXXVII; also methods for obtaining blood and injecting intramuscularly. The subject of Blood Transfusion is considered in Chapter XLII.

Reactions Following the Administration of Vaccines, Sera, and Non-specific Agents.—These have been discussed in previous chapters, and the physician employing these substances in the treatment of disease should be familiar with the reactions that may be elicited, the dangers, and contraindications. The reactions that may follow the injection of serum are described on p. 839; skin tests for anaphylactic sensitization are described on p. 680 and methods for desensitizing on p. 722.

Reactions following the injection of vaccines are described on p. 762 and of non-specific substances on p. 881.

TREATMENT OF ABSCESSSES (FURUNCULOSIS) AND CARBUNCLES

Ordinary abscesses or furuncles are usually caused by *Staphylococcus aureus*; stitch abscesses or similar superficial lesions may be caused by *Staphylococcus albus* and occasionally by *Staphylococcus citreus*. Carbuncles are usually caused by *Staphylococcus aureus* and occasionally by *Streptococcus pyogenes*.

When numerous abscesses occur the urine should be examined for sugar; not infrequently the blood-sugar is found higher than normal, even though sugar is not found in the urine.

Immunity in Staphylococcus Infections.—Man and practically all of the lower animals are subject to staphylococcus infections. Most of the lower animals and notably the rat, appear, however, to possess a greater degree of natural immunity than man and can withstand very large injections of virulent organisms.

Natural immunity is largely dependent upon intact epithelial barriers and the secretions. Any agent producing lowered resistance of the skin, mucous membranes, and the cutaneous glands greatly increases susceptibility to staphylococcus infections. Practically every organ and tissue is vulnerable to infection.

Phagocytosis aided by opsonins constitute the chief means of defense and recovery when the cocci have gained access to the deeper tissues. The

blood and serum possess staphylococidal activity, but phagocytosis is undoubtedly of more importance.

Immunity to staphylococcus infections may be raised by appropriate immunization by means of vaccines; this immunity is principally due to the production of specific immune opsonins. But the duration of staphylococcus immunity after active immunization or after recovery from the disease tends to be of short duration. For this reason the writer advocates a second course of vaccine injections within four to six months after the first in the treatment of furunculosis, even though the patient is free of abscesses at this time.

Vaccine Treatment of Staphylococcus Infections.—Stock staphylococcus vaccine frequently yields as good results as autogenous vaccine and may be employed while the latter is being prepared. Cultures of pus may be made on any suitable medium. There is considerable evidence indicating the existence of serologic strains among the staphylococci, which renders advisable the use of autogenous vaccine whenever it can be properly prepared.

The vaccine may contain 2,000,000,000 cocci per cubic centimeter. The first dose may be 0.1 c.c. or 200,000,000. The injections should be subcutaneous at intervals of seven days and never less than five days. Each succeeding dose may be increased by 0.1 or 0.2 c.c. until 1 c.c. is being given at one time.

In infants and children under six years of age the vaccine may contain 500,000,000 per cubic centimeter, the first dose being 0.1 c.c. and gradually increased in the same manner. In children six to twelve years of age the vaccine may contain 1,000,000,000 per cubic centimeter. Children over twelve years may receive the adult doses.

As a general rule four to ten injections are required; sometimes one or two suffice. In chronic furunculosis I believe it is a good plan to give two or three more injections four to six months later in order to reinforce the immunity, even though the patient is free of furuncles at that time.

As a general rule slight soreness follows at the site of injection and occasionally a mild constitutional reaction. With the first few doses a slight focal reaction may develop characterized by tenderness of the lesions with increased discharge of pus; in chronic furunculosis these focal reactions are desirable, providing care is taken not to render them severe by excessive doses.

The results of vaccine treatment of recurring furuncles and carbuncles have been generally very good. This is especially true of deep-seated lesions. Superficial lesions and particularly those produced by *Staphylococcus albus* are frequently not improved by this therapy. In chronic furunculosis of infants particularly good results may be obtained. In both adults and children recurrent furunculosis and the development of carbuncles are usually accompanied by underweight, anemia, and other constitutional conditions which lower staphylococcus immunity and require appropriate attention.

Serum Treatment of Staphylococcus Infections.—While several attempts have been made to treat staphylococcus infections with an immune serum, the investigations have been too few and too brief to warrant a statement in regard to the value of serum therapy in these infections. Thomas¹ prepared a serum by immunizing a ram with 18 different strains of *Staphylococcus pyogenes aureus*, and reported good results in the treatment of 28 cases of furunculosis and carbuncles.

¹ Jour. Amer. Med. Assoc., 1913, lx, 1070.

It is probable that, with more extensive use of antistaphylococcus serum, its value will be proved, especially in the treatment of severe furunculosis of infants as well as of adults, when the low general vitality of the patient contraindicates the use of a bacterial vaccine. With extended use it may also be found to be of benefit in staphylococcus bacteremia, osteomyelitis, arthritis, carbuncle, and other severe infections.

The activity of the serum is probably largely dependent upon the presence of bacteriotropins and antitoxins, the former promoting phagocytosis and the latter neutralizing the staphylolysins or hemotoxic poisons produced by staphylococci.

Non-specific Treatment of Staphylococcus Infections.—Turpentine injections for the purpose of producing "fixation abscesses" and the consequent absorption of these products, were introduced by Fochier,¹ who injected 1 c.c. subcutaneously or intramuscularly. Cerioli² has modified this plan by injecting 1 c.c. of a mixture composed of 16 parts eucalyptol and 4 parts turpentine. The abscesses are quite large and more recently French and German clinicians have modified these methods and are now injecting intramuscularly at frequent intervals a few drops of a mixture of 4 parts of turpentine in 16 parts of sterile olive oil.

Milk injections have sometimes yielded particularly good results. Ordinary pasteurized or raw milk is boiled for five to ten minutes and when cooled 3 to 5 c.c. are injected intramuscularly. As a general rule a mild fever and leukocytosis develop in six to eight hours which subside in twenty-four to forty-eight hours. The injections may be given at intervals of seven to ten days.

TREATMENT OF SEPTIC WOUNDS AND SINUSES; LYMPHADENITIS

Bacteriology.—A variety of aërobic and anaërobic bacteria have been found in the septic wounds of the war, including the chronic sinuses, with and without sequestra. Among the aërobes, *Streptococcus pyogenes* has been most frequent and most important; *staphylococci*, *Bacillus coli*, *B. proteus*, *B. pyocyaneus*, and numerous strains of coliform bacilli have also been found and usually in mixed culture. Of the anaërobic spore-bearing bacilli, *B. tetani*, *B. perfringens* (*B. aërogenes capsulatus*, *B. welchii*), *B. malignant edema* (*vibrio septique*), and *B. oedematiens* were the most common. One of the most remarkable lessons of the bacteriology of war wounds was the long periods of time in which bacteria and particularly the anaërobes remained in the tissues, and especially in sinus cases with sequestra; tetanus and gas gangrene developed many weeks after trivial wounds and operations, and healed wounds frequently broke down with severe sepsis as the result of too early exercise.

Vaccine Treatment of Septic Wounds.—A great many cases of septic wounds have been treated with vaccines during the recent war, and especially by the English surgeons; it is exceedingly difficult, however, to evaluate the results because of widely divergent opinions. Certainly the results have not been impressive—temperatures did not fall by crisis nor discharges cease as if by magic, and a decision whether vaccines have done any good relies mainly upon clinical impressions. Sir Berkeley Moynihan³ believes "that a case has been made out for the trial of vaccines in appropriate cases and in suitable conditions," and is thoroughly convinced of the great value of streptococcus vaccine in serious wounds.

¹ Progrès méd., 1892, 15, 355.

² Gazz. d. osp. e d. clin., 1915, 36, 721.

³ Lancet, 1916, 1, 333.

It is the general consensus of opinion that vaccines had none or but slight direct influence upon the bacterial flora of septic wounds and sinuses; the basis for vaccine treatment rests upon the principle of producing antibodies and thereby raising the antibacterial power of the serum, providing for its access to the wound as with the use of Wright's¹ hypertonic saline solution which promotes the flow of lymph into the wound, and providing proper drainage and dressings. Wright² claims that this lymph not only brings into the wound bacteriotropic and bactericidal substances, but promotes tryptic activity resulting in digestion of sloughs and aiding in the cleansing of the wound. Wright has also observed that the pus of these sloughing wounds affords a good medium for numerous varieties of bacteria and the gas gangrene group in particular (the *serosaprophytes*), whereas in fresh lymph—more or less unaltered blood fluids—only a few of these, of which the streptococci and staphylococci are most important, are able to grow (the *serophytes*).

Vaccines are prepared of the aërobic bacteria only, and especially of streptococci; mixed vaccines are advisable if staphylococci, *Bacillus proteus*, *B. coli*, and *B. pyocyaneus* are also present. Such vaccines are given as soon as possible in the attempts to increase the resistance to general constitutional disturbances subsequent to surgical manipulation of the infected tissues, and to prevent the spread of bacterial infection of the tissues in the neighborhood of the wound; Goadby³ has advised at least four injections over a period of two weeks preceding operation, and in a large series of cases found that this practice eliminated "flares" and greatly reduced the percentage of secondary hemorrhages due to septic infection. Tidy⁴ and Shera⁵ and Swan⁶ have had similar experiences; the presence of anaërobic bacteria was ignored, provided the patient received these immunizing inoculations and adequate drainage was provided.

Prophylactic immunization among soldiers with antiseptic vaccine prior to exposure to injury has not been tried on a large scale, although strongly endorsed by Sir Berkeley Moynihan, Sir Almoth Wright, and Sir Watson Cheyne; stock vaccines prepared of strains of bacteria from war wounds have been commonly employed in individual cases while autogenous vaccines were being prepared.

The doses should not be too large; the administration of a streptococcus vaccine may begin with 50,000,000 and gradually increased; mixed vaccines may begin with 25,000,000 of each of the important aërobic bacteria present, followed by increasing doses every three to five days over a period of two to three weeks. Operations should not be attempted for at least two days after the last dose.

TREATMENT OF STREPTOCOCCUS INFECTIONS AND SEPTICEMIA WITH SPECIAL REFERENCE TO CELLULITIS, PUERPERAL SEPSIS, AND ENDOCARDITIS.

Kinds of Streptococci in Relation to Biologic Therapy.—There appear to be many saprophytic and non-pathogenic as well as pathogenic varieties of streptococci. Owing to variations in morphology, biologic characters, and virulence it has so far been impossible to make a satisfactory classification. The relation of the pneumococcus to the group has not been fully

¹ Lancet, June 23, 1917.

² Lancet, April 29, 1919, Brit. Med. Jour., 1915, 1, 625; *ibid.*, 1915, 2, 629, 717.

³ Lancet, 1916, 2, 89.

⁴ Lancet, 1915, 2, 329.

⁵ Vaccines and Sera in Military and Civil Practice, Hodder and Stoughton, 1918.

⁶ Lancet, November 18, 1916.

decided. It was first thought that the streptococci of erysipelas, puerperal sepsis, scarlet fever, etc., belonged each to a different species, but it is now generally believed that the slight differences among the majority of streptococci from these diseases were but acquired variations of organisms derived from the same species. Differences exist among streptococci, but these are not constant according to the source (disease).

Some streptococci are able to produce definite hemolysis, and this type is probably the original *Streptococcus pyogenes* of Rosenbach, since designated by Rolly as *Streptococcus hemolyticus* and by Blake¹ as *Streptococcus hemolysans*. Dochez, Avery, and Lancefield² have recently shown that at least four biologic types exist among these as shown by agglutination and protection tests. A second group does not produce hemolysis; some of these produce methemoglobin including *Streptococcus viridans*, and others do not, designated as *Streptococcus nonhemolyticus*.

The group of streptococci is, therefore, very large and indicates that in vaccine therapy autogenous vaccines should be prepared in order to make sure that the same strain or strains are being employed. In serum therapy the antistreptococcus serum should be polyvalent, but probably better results will be obtained by the immunization of horses with streptococci from the one disease instead of preparing one serum for all streptococcus infections.

Immunity in Streptococcus Infections.—Human beings and most of the lower animals possess a fairly well-marked degree of natural immunity to streptococci. Of the lower animals, young rabbits are particularly susceptible. Highly virulent streptococci, however, readily overcome the barriers to infection, and when gaining access to the lymph and blood are able to proliferate rapidly, produce virulent poisons, and prove very dangerous infections. In other words, natural defenses are readily overcome by highly virulent streptococci.

Natural immunity is largely dependent upon intact epithelial barriers, the secretions and phagocytosis. *Streptococcus* poisons are in the nature of aggressins, that is, repel leukocytes and retard phagocytosis. Recovery from infection is largely brought about by the production of anti-aggressins and opsonins aiding phagocytosis, and probably, to a lesser extent, by the production of agglutinins and streptococcidal lysins in the blood.

Immunity to streptococci is built up very slowly. One attack does not confer more than a transient resistance and, indeed, as in recurring erysipelas, there may be no evidences of local or general acquired immunity at all even to the streptococci of the same disease.

Vaccine Therapy of Streptococcus Infections.—There is a great difference of opinion on the subject of using vaccines in acute streptococcus infections. Probably the majority of physicians believe that the patient should not receive this extra stimulation. In *streptococcus cellulitis* and *lymphadenitis* the results of judicious vaccine therapy is, however, usually very satisfactory. In *puerperal streptococcus endometritis* the writer has observed good results when the vaccine injections were started early instead of late in the infection. In *acute streptococcus endocarditis* with recovery of the micro-organisms in blood-culture, as likewise in streptococcus bacteremia (septicemia), Barr and Bell,³ Thompson,⁴ Rosenow,⁵ and others have recorded encouraging results. Horder,⁶ Billings,⁷ and others, however,

¹ Jour. Med. Res., 1917, 36, 99.

² Jour. Exper. Med., 1919, 30, 179.

³ Lancet, February 23, 1907, 499.

⁴ Amer. Jour. Med. Sci., 1909, 138, 169.

⁵ Jour. Amer. Med. Assoc., 1910, 55, 1719.

⁶ Quart. Jour. Med., 1909, 2, 289.

⁷ Arch. Int. Med., 1909, 4, 409.

have reported negative results. It would appear that the acute types of endocarditis are more favorable for vaccine therapy than the slow, insidious types associated with progressive anemia and due to streptococci of low-grade virulence.

The vaccine in small doses cannot be said to be primarily toxic; an injection does not add to the amount of toxic substances to be combated. It is largely a question of whether or not benefit is to be derived from extra stimulation of antibody producing tissues and whether these tissues are sensible to this extra stimulation and capable of a beneficial response. My decision in individual cases is based upon the general condition of the patient and the duration of the disease. If the patient is fairly robust I believe that small doses of vaccine are advisable in the *early* stages; if the patient is anemic and emaciated, as in the later stages, vaccine therapy is contraindicated.

Stock vaccine may be employed while attempts are being made to recover the infecting streptococcus for the preparation of an autogenous vaccine. The vaccine should contain approximately 500,000,000 per cubic centimeter; the first dose is 0.1 c.c. or 50,000,000. Injections should be subcutaneous, repeated every five days and in gradually increasing amounts.

Serum Treatment of Streptococcus Infections.—The acute character of streptococcus infections and their relative frequency and severity have made them the subject of numerous efforts on the part of various investigators toward developing an efficient serum therapy. To Marmorek belongs the credit of first attempting, in 1895, to prepare a curative serum on a large scale. Since then Aronson, Tavel, Krumbein, Moser, Meyer-Ruppel, Menzer, and others have prepared immune serums with various cultures and according to various methods. While many antistreptococcus serums will show undoubted protective value, especially against their homologous cultures as tested in experimental animals, the general opinion regarding their curative value in streptococcus infections of man have been conflicting and, as a rule, unfavorable. Occasionally the rapid improvement of a patient following an injection of the serum would indicate that it has proved beneficial, and the same is occasionally true of a particular group of infections treated with a specially prepared serum. The tendency of acute streptococcus infections to end spontaneously by crisis must, however, be borne in mind, and the good results observed in individual cases may be coincident with, rather than the result of, the administration of the serum.

Several causes for the failure of antistreptococcus serum therapy are now understood, and if these can be eliminated the value of this form of therapy will be greatly augmented.

1. *The serum should be given in large doses, and by intramuscular and intravenous injection.* In a true streptococcic infection the cocci are likely at some time to be found in the blood-stream, and an attempt to destroy these organisms or to limit a local infection by injecting 10 c.c. of serum in the subcutaneous tissues is almost sure to result in failure. As in the serum treatment of pneumonia, at least 100 c.c. of serum should be given intravenously and the dose repeated if necessary.

2. *The serum should be used as early in the disease as possible instead of waiting until the patient has become moribund.* In puerperal sepsis and scarlet fever, for instance, the question of serum therapy should be considered early, for when properly administered the serum will at least do no harm and may prove efficacious. In order to determine the value of the serum a bacteriologic diagnosis should always be attempted, especially by means of blood-cultures obtained by placing from 2 to 5 c.c. of blood in a flask

containing at least 100 c.c. of dextrose broth just prior to injecting the serum.

3. *The serum should be polyvalent.* Marmorek maintains that all streptococci are alike, and he has, accordingly, prepared his serum from a single highly virulent strain. Thus far no adequate methods for classifying these organisms have been discovered, but it is likely that future researches will show that streptococci from different infections possess different immunologic characters similar to the variations observed among the pneumococci causing lobar pneumonia. If this is found to be true, under these conditions, a similar serum treatment, while complicated, is likely to prove valuable in the treatment of streptococcus infection. Tunncliffe¹ has recently reported that the serum of a sheep immunized with hemolytic streptococci from the throat in the acute stage of scarlet fever showed agglutinins, opsonins, and protective power highly specific for these micro-organisms. For the treatment of streptococcus infection in scarlet fever the serum should be prepared of numerous strains isolated from patients having this disease. What has just been said is also true of the other three infections so frequently streptococcal, namely, puerperal sepsis, phlegmonous cellulitis, and ulcerative endocarditis. If not these four, at least two antistreptococcus serums should be available; one for scarlet fever and the second for other infections; both, and especially the latter, should be prepared by immunizing horses with a large number of various strains.

Mode of Action of Antistreptococcus Serum.—Virulent streptococci exert a powerful negative chemotactic influence upon leukocytes, repelling them and effectively resisting phagocytosis for varying periods of time. The early researches of Bordet showed that antistreptococcus serum neutralizes this influence and promotes phagocytosis. Since then numerous investigators have supported Bordet's findings, so that it may be accepted as true that one of the chief antibodies in antistreptococcal serum is of the nature of a *bacteriotropin* or immune opsonin. A potent serum also contains an *antitoxin*, as may be shown experimentally by neutralization of the hemotoxic poison of streptococci, and also clinically, when the rapid subsidence of fever and general improvement of the patient are probably due, in part, to neutralization of streptococcal toxins. Thus far the presence of *bacteriolysins* has not been definitely proved, although they may be present and operative *in vivo*. I have found that antistreptococcus serum contains an antibody capable of fixing complement with streptococcus antigens.² It may be stated, therefore, that the action of antistreptococcus serum is dependent primarily upon bacteriotropins, and secondarily upon antitoxins and, possibly, bacteriolysins.

Preparation of Antistreptococcus Serum.—Some differences of opinion have been expressed regarding the advisability of passing cultures that are being used for purposes of immunization through a lower animal in order to increase their virulence. For example, the unsatisfactory results that have followed the use of Marmorek's serum have been ascribed not only to the fact that it is monovalent, but also to possible alteration of the strain in its biologic characteristics by animal passage, so that its virulence for the human being was diminished or lost, and, accordingly, while the antiserum is protective for the animals through which the passage has been conducted, it is inactive for the human being. Tavel, Krumbein, and Paltauf have prepared polyvalent serums with different strains from human infections without animal passage. Menzer has prepared a serum with strains of cocci derived from acute rheumatic fever, and Moser with strains obtained from scarlet fever, which have also not been passed through animals. Aronson has attempted a combined procedure, making use of passed and unpassed cultures conjointly, and this appears to be the method of choice. In other words, those who prepare antistreptococcus serums should use as many

¹ Jour. Amer. Med. Assoc., 1920, 74, 1386; *ibid.*, 1920, 75, 1339.

² Arch. Int. Med., 1912, ix, 220.

fresh strains as possible, and in several of the older cultures the virulence should be increased from time to time by passage through animals.

In preparing the serum young and healthy horses should be used. The injections should first commence of dead cultures given subcutaneously, then of autolysates, and finally of living cultures administered intravenously. Occasionally severe local and general reactions are observed, and the whole procedure should be conducted under careful supervision.

First Method.—Cultures are grown on a solid medium, and an emulsion and autolysate prepared as described for immunizing with meningococci. Begin by injecting subcutaneously 5 c.c. of emulsion heated to 60° C. for one hour, and increasing the dose each week by 5 c.c. until 100 c.c. are given at one time. If the reactions are mild (general and local), the doses may be increased more rapidly. Then begin with 2 c.c. of living culture and increase the dose each week. When a dose of 10 c.c. is reached, inject with autolysate and living cultures alternately, gradually increasing the dose until a dose of 50 c.c. is reached. Living cultures are then given intravenously and the dose rapidly increased until 100 c.c. and more are given at one time. Intravenous injections are not infrequently tolerated better than subcutaneous injections. The horses may be bled several times during the course of immunization and their serums tested. When the serum is to be used therapeutically, the animals should not be bled in less than from ten to fourteen days after the last injection was given. In view of the large doses required, a concentrated serum is advisable (Heinemann and Gatewood¹). Since trikresol has an inhibiting influence on phagocytosis (Weaver and Tunnickliff²) the minimal quantity (0.2 per cent. or less) should be used.

Second Method.—Kolle prepares a polyvalent serum with cultures derived from cases of erysipelas, puerperal sepsis, scarlet fever, etc. Their virulence is increased from time to time by passage through rabbits.

Horses are used for immunization purposes and all injections are given intravenously at intervals of a week. Cultures are grown on test-tubes containing a solid medium, and immunization is started with half a culture, heated. The dose is increased each week with an additional culture until it equals 16 cultures. Then living and killed cultures are mixed, giving in one week 2 living and 14 killed cultures and so on until 10 living and 6 killed cultures are given at a single dose. The horses are bled two weeks after the last dose is administered. Caulfield³ has recently described new methods of promising value.

Standardization of Antistreptococcus Serum.—There is at present no single satisfactory method for standardizing these serums, although a satisfactory method is a desideratum for testing serums placed on the market. In order to obtain an approximate idea as to the value of a serum, the following tests may be employed:

1. *Protective Value.*—At the Serum Institute in Vienna a passed culture (one used in the process of immunization) is selected, and that dose which will kill a mouse at the expiration of or just preceding the end of four days is regarded as a single lethal dose. In testing an antiserum ten times this quantity of culture is used with decreasing doses of immune serum injected twenty-four hours previously or simultaneously. A normal serum is one of which 0.1 c.c. will afford protection, and 1 c.c. of such a serum is said to be one immunity unit, *i. e.*, it affords protection against 1000 lethal doses of culture. For conducting these protection tests the same technic and standard described for the standardization of antipneumococcus serum may be employed.

2. *Bacteriotropic Value.*—The technic of Wright or Neufeld may be employed with a virulent culture and human leukocytes. Weaver and Tunnickliff have observed better results when using 1 part of immune serum reactivated with 9 parts of fresh guinea-pig serum. The technic and standard for conducting these tests may be similar to that employed by Evans in the Hygienic Laboratory for the testing of antimeningococcus serum.

3. *Agglutination Tests.*—These may be conducted in the same manner as tests employing antipneumococcus or antimeningococcus sera; a macroscopic technic is employed with an incubation of sixteen to eighteen hours at 55° C. A satisfactory serum should have a titer of 1 : 400 or higher.

4. *Complement-fixation Tests.*—These tests may be employed with the bacterial emulsion or autolysate used in immunization as the antigen.

Preservation.—The serum should be kept in a cool, dark place. After a few months it loses some of its protective value, and much of it on the market is worthless.

Administration of Antistreptococcus Serum.—*It must be emphasized here that, in order to obtain the best results, antistreptococcus serum must be given intravenously.* In an adult patient with a severe general infection from 30 to 100 c.c. of serum, diluted with an equal amount of sterile normal

¹ Jour. Infect. Diseases, 1912, 10, 416.

² Jour. Infect. Diseases, 1911, 9, 130.

³ Jour. Path. and Bacteriol., 1916-17, 21, 28.

salt solution, may be given in one dose. If improvement follows, subsequent doses should be given subcutaneously or intramuscularly in order to prolong the action of the serum. If no improvement follows in from twelve to twenty-four hours, or if an acute exacerbation sets in, a second dose should be given intravenously. Since the various manufacturers use different cultures in the preparation of these serums, it would be well to *use a different brand of serum if the first does not exert a beneficial effect*. In patients with severe infections the activity of the serum may be enhanced by adding, just before injection, 5 c.c. of fresh sterile guinea-pig serum to each 50 c.c. of the immune serum; for example, Tunncliffe has shown that sheep anti-streptococcus serum for scarlet fever rapidly lost its opsonic and protective power, which was restored by the addition of fresh normal sheep serum.

Value of Antistreptococcus Serum.—Although the serum has been in use for almost twenty-five years, the true exact value of the remedy has not as yet been estimated. It may be stated that a carefully prepared and properly administered serum *will do no harm and may do good*, and that its use should form a part of the treatment of severe streptococcal infections.

In some cases of *wound infections* with severe cellulitis and septicemia the serum may at times exert a most pronounced and happy effect. In other cases, and especially in those in whom the cocci are found in the blood, even repeated injections may be of no value.

In *puerperal sepsis* and *endocarditis* of streptococcal origin the results of serum treatment have not been uniform, but are generally unfavorable. If serum is administered at all, it should be given early, in large doses, and intravenously. Not all cases of puerperal sepsis are streptococcal, and while the physician may not be justified in withholding serum until a bacteriologic diagnosis has been made, this factor must be considered when estimating the value of a serum.

Blood Transfusion in the Treatment of Streptococcus Septicemia.—*Immunized Blood.*—In Chapter XLII this subject is considered in more detail. Occasionally in severe, prolonged streptococcus infections with anemia, as in puerperal sepsis, ulcerative endocarditis and cellulitis, the transfusion of 500 c.c. of compatible human blood turns the tide in favor of recovery. One drawback is the possible harmfulness of the reaction that may follow transfusion.

If time permits it may be advisable to immunize the donor beforehand, as recently reported by Dick.¹ The donor may receive three injections of heat killed vaccine on successive days of 1,000,000,000, 2,000,000,000, and 3,000,000,000. Transfusion may be done a week later, injecting 50 to 100 c.c. of blood and repeating again next day or giving 500 c.c. at one time. Wright² has recently stated that the transfusion of plain blood may be of no avail because more and better pabulum is supplied the micro-organisms. His experiments have suggested the practical utilization of adding vaccine to the blood and after a suitable interval of about three hours injecting the whole into the patient. Wright has observed that this "immunization *in vitro*" is non-specific, enhances the bactericidal activity of the serum, and calls the method "*immuno-transfusion*." In a case of streptococcus wound sepsis Wright drew off 1 liter of blood from a compatible donor into a paraffin coated receptacle, added 1,000,000 killed staphylococci, and later injected this mixture intravenously with a successful result. Whether or not citrated blood may be used is not stated and the method requires further trial before an opinion of its value may be given.

¹ Jour. Amer. Med. Assoc., 1922, 78, 1192.

² Lancet, March 29, 1919.

Leukocytic Extracts, Peptone, and Other Non-specific Agents in the Treatment of Streptococcus Infections.—Leukocytic extract may be injected subcutaneously or intramuscularly in doses of 10 to 15 c.c. daily for several days.

Gow¹ has employed intravenous injections of 10 per cent. solutions of Witte's peptone in the treatment of septicemia; 8 to 10 c.c. are injected very slowly by means of a very fine needle (No. 28) and repeated every other day in doses increased by 2 c.c. until 16, 18, or 20 c.c. are given at one time. Gow makes no extravagant claims for this method, although he thinks it is a valuable adjunct to treatment. Nolf² has likewise employed intravenous injections of peptone in the treatment of streptococcus, staphylococcus, and pneumococcus septicemias, and claims good results.

Lüdke³ has employed injections of deuterio-albumose. Lindig and Arweiler⁴ have used injections of casein. Intramuscular injections of milk have been employed by Bianchi,⁵ but the results have not been particularly encouraging when streptococci were present in the blood.

TREATMENT OF ERYSIPELAS

Erysipelas is also a streptococcus infection, and much that has been stated in the preceding paragraphs applies to its treatment with biologic agents.

Immunity in Erysipelas.—Disposition to this disease is wide-spread, but susceptibility is specially marked in the cases of recently delivered women, after injuries, burns, and surgical operations. Sometimes infection apparently begins within the nose and spreads to the skin.

One attack does not prevent subsequent attacks; indeed, the patient may be left even more susceptible. The disease has been experimentally produced in the same area of skin many times, indicating that even a local tissue immunity is not engendered. As previously stated in discussing immunity to streptococcus infections in general, natural resistance is largely due to phagocytosis. In the mechanism of recovery, phagocytosis aided by specific opsonins are apparently of most importance.

Vaccine Treatment of Erysipelas.—Vaccine treatment has generally failed to influence the disease and probably most physicians are opposed to the use of vaccines in acute erysipelas; it cannot be denied, however, that when vaccines have been employed *early* in the infection, that not infrequently the lesions and symptoms have rapidly subsided. Owing to the self-limited character of erysipelas it is not always easy, however, to evaluate the worth of a therapeutic measure. Because of the difficulty and delays in obtaining the streptococcus in pure culture, it is necessary to employ a stock streptococcus vaccine for a time at least. During the acute stage the doses should be small, beginning with 0.1 c.c. of a vaccine containing 500,000,000 killed streptococci per cubic centimeter; the injections should be given subcutaneously every five days and in gradually increasing amounts.

In chronic and recurring erysipelas vaccine therapy is more strongly indicated; the dosage and intervals of injections should be as stated above until 1 c.c. of vaccine is being given at one time.

Serum Treatment of Erysipelas.—Antistreptococcus serum has been frequently employed in the treatment of erysipelas, with indifferent results.

¹ Brit. Med. Jour., 1920, 2, 268.

² Jour. Amer. Med. Assoc., 1919, 73, 1579.

³ Berl. klin. Wchn., 1920, 47, 344.

⁴ Therap. Halbmonatsch., 1920, 34, 470.

⁵ Riforma med., 1920, 36, 1026.

The usual procedure has been to inject 10 to 20 c.c. of the serum subcutaneously and these amounts were too small. In severe spreading infections accompanied by high fever, considerable edema, and delirium, serum should be given, but as early as possible, intravenously and in dose of 50 to 100 c.c., repeated the following day if necessary. Administered in this manner beneficial effects are to be expected. Jez¹ has reported favorably upon the treatment of erysipelas by subcutaneous and intravenous injections of 10 to 15 c.c. of the patient's own serum. Kaiser² has reported a case successfully treated by injections of whole blood from a convalescent donor.

Leukocytic Extract and Other Non-specific Agents in the Treatment of Erysipelas.—Lambert,³ Hess and Dwyer,⁴ and others have reported favorably upon the subcutaneous injection of 10 to 15 c.c. of leukocytic extract once a day for several days in the treatment of acute erysipelas.

Petersen⁵ has reported that the intravenous injection of 0.5 to 2 c.c. of a 2 per cent. solution of proteoses has yielded very satisfactory results; also the administration of milk and typhoid vaccine. Holler⁶ has employed intravenous injections of deuterio-albumose, but the doses must be small in order to avoid severe general reactions which are readily induced (not over 0.1 to 0.5 c.c. of 10 per cent. solution). Blumenau⁷ has employed nuclein injections; sodium nucleinate may be injected subcutaneously in dose of 0.5 gm.

Schmidt⁸ and others have employed intramuscular injections of 5 to 10 c.c. of market milk boiled for ten minutes and cooled.

Normal horse-serum and diphtheria antitoxin have been employed, especially by the French clinicians, in dose of about 10 to 20 c.c. by subcutaneous injection.

My impression is that these non-specific agents are especially indicated in chronic and recurrent erysipelas; great care must be exercised in dosage because of the chances of eliciting severe general reactions.

TREATMENT OF SCARLET FEVER

The etiology of scarlet fever being unknown, the only true form of specific treatment is by means of injections of serum from convalescent cases. One attack of the disease generally confers an immunity for life, and immunity principles are doubtless in the serum, at least for a short space of time after recovery.

Streptococci are known to have a very close relationship to the disease; probably they are the most important bacteria of secondary infection. These streptococci are usually of the hemolytic variety, are responsible for the severe throat lesions and adenitis, and may be more or less specific for scarlet fever, as indicated by recent investigations by Tunnickliff, to which reference has been previously made.

Vaccine Treatment of Complications of Scarlet Fever.—This refers almost solely to the use of vaccines in the treatment of complications, as suppurative adenitis, otitis media, etc. Streptococci and Staphylococcus aureus are the usual findings in the acute cases; in chronic infections other

¹ Wien. klin. Wchn., August 31, 1901.

² Arch. Pediat., 1915, 32, No. 7.

³ Amer. Jour. Med. Sci., 1909, 137, 506.

⁴ New Jersey Med. Rec., 1913, lxxxi, No. 11.

⁵ Protein Therapy and Non-specific Resistance. MacMillan Co., 1922, 191.

⁶ Med. Klinik, 1917, 13, 1038.

⁷ Ztschr. f. Immunitätsf., Ref., 1911, 3, 1075.

⁸ Med. Klinik, 1916, 12, 171; *ibid.* 1920, 16, 691.

bacteria may be found. I believe it is always a good plan to culture the pus from these lesions and hold an autogenous vaccine in readiness for later use in case of necessity.

While streptococcus vaccines have been advocated as a means of prophylactic immunization against scarlet fever, they have not proved of value in the treatment of the disease.

Treatment of Scarlet Fever with Antistreptococcus Serum.—In severe anginose or malignant *scarlet fever* large doses of serum from horses especially immunized with strains of streptococci from scarlet fever patients have, on the whole, yielded favorable results. Not all cases of severe scarlet fever, however, are due to secondary streptococcal infections: those patients who are overwhelmed and prostrated at the very outset are probably intoxicated with the true scarlatinal virus, whatever that may be, and such cases are not likely to be benefited by serum treatment. The patients most likely to improve under serum therapy are those who become severely ill after the onset of the disease and the appearance of the eruption.

The serum should be prepared by the immunization of horses with strains of streptococci from scarlet fever cases. Very probably it has no specific effect upon the scarlatinal virus, but solely upon the secondary streptococcus infection and should be employed in severe infections for this purpose. In Russia, where scarlet fever has a high morbidity and mortality, Moltchankoff¹ has found that serum treatment reduced the mortality from 47.4 to 16.1 per cent.

For adults the dose of antistreptococcus serum should be 50 to 100 c.c. by intravenous injection and repeated twelve hours later and again the next day. Children may receive 25 to 50 c.c. by intravenous injection if possible; otherwise 25 to 50 c.c. by intramuscular injection, repeated daily for three or four days if necessary.

Treatment of Scarlet Fever with Convalescent Serum and Blood.—Weisbecker² in 1897 first employed the sera of persons convalescent from scarlet fever in the treatment of acute cases; Huber and Blumenthal,³ von Leyden,⁴ Rumpel,⁵ Scholtz,⁶ Reiss and Jungman,⁷ Koch,⁸ Rowe,⁹ Zingher,¹⁰ and others have reported favorably upon the treatment of scarlet fever and particularly severe infections, with injections of serum from persons who have recovered from this disease.

Kling and Widfelt¹¹ in 1918 reported on the use of convalescent serum in the treatment of 237 cases. Of these, 17.7 per cent. died, while in a corresponding group of 91 severe cases in which no serum was given, the mortality was 70 per cent. Of those receiving serum on the second day, 93.7 per cent. recovered; with each day's delay the mortality increased, until by the sixth day only 50 per cent. recovered. They believed that severe toxic cases were benefited by the convalescent serum. They found the serum from persons who had mild attacks as effective as that from persons who had severe cases, but they also observed a variation in the effectiveness of sera from different individuals.

¹ Jahrb. f. Kinderh., 1907, lxvi, 503.

² Ztschr. f. klin. Med., 1897, 32, 188.

³ Berl. klin. Wchn., 1897, xxxiv, 671.

⁴ Deutsch. Archiv. f. klin. Med., 1902, lxxiii, 616.

⁵ Münch. med. Wchn., 1903, 50, 38.

⁶ Fortschr. d. Med., 1903, 21, 353.

⁷ Deutsch. Archiv. f. klin. Med., 1912, cxii, 70.

⁸ Münch. med. Wchn., 1913, lx, 2611; Deutsch. med. Wchn., 1915, xli, 372.

⁹ Med. Klinik., 1913, ix, 1978.

¹⁰ Jour. Amer. Med. Assoc., 1915, 65, 875.

¹¹ Hygiea, 1918, 80, 2.

Barker,¹ Weaver,² Bode,³ Synnott,⁴ Glaser,⁵ Schultz,⁶ and others have likewise reported most favorably upon this treatment of severe toxic cases of scarlet fever. An initial fall of temperature usually occurs with improvement of the restlessness, delirium, and great improvement of the general condition of the patient.

Reiss and Jungman obtained the serum from persons about the third week of the disease and injected 50 to 100 c.c. intravenously. Koch emphasizes the necessity of starting the treatment as early in the disease as possible and injecting large doses of the serum intravenously. Rowe was unable to convince himself that there were any different effects in cases treated with normal and with convalescent serum; Koch suggests that the convalescent serum be reserved for the gravest cases, and that otherwise normal human serum be used. Zingher has used intramuscular injections of whole citrated blood, administering from 2 to 8 ounces in a series of injections at close intervals. Normal human blood was also found of value in the treatment of a group of later septic cases, seen from the fifth to the eighth day of the disease. It is apparent that these methods of treatment are worthy of further trial, and particularly in the treatment of severe or anginose cases of the disease.

The *technic* is very simple and the method of treatment particularly applicable in hospitals for the treatment of scarlet fever. Donors are selected after the second week of the disease, and preferably from among adolescents or adults who show no evidences of tuberculosis. From each person 30 to 100 c.c. of blood may be obtained under aseptic precautions and the serum carefully separated, submitted to the Wassermann test, cultured for sterility, and preserved with 0.2 per cent. tricresol in sterile containers in a refrigerator. In young children an intravenous injection may not be possible unless one of the larger veins, as the external jugular or longitudinal sinus, is selected. It is advisable to inject the serum as early in the disease as possible in large doses (20 to 50 c.c. for a child seven years of age) and, preferably, by intravenous injection. The injections appear to be harmless and no special precautions as to the presence of hemagglutinins or hemolysins appear necessary. Otherwise the serum may be injected intramuscularly in the gluteal region, and in the absence of specific convalescent serum, normal human serum collected in the same manner may be injected. As a general rule three or four injections are required at intervals of six to twelve hours to influence the disease, and particularly the severe infections.

In using whole blood the method employed by Zingher is very satisfactory. Two c.c. of a sterile 10 per cent. solution of sodium citrate in normal salt solution is placed in a sterile 100-c.c. bottle; 2 ounces of blood are collected, added, and briefly shaken; the blood is now ready for intramuscular injection. Wassermann reactions should be made beforehand on all possible donors so that the proper persons may be selected. In private practice the physician may take the blood from either one of the parents or close relatives.

Weaver has injected the serum intramuscularly in amounts of from 60 to 90 c.c. A second dose was given after twenty-four hours when the first was not followed by satisfactory improvement or when improvement was followed by a tendency to relapse.

¹ Arch. Pediat., 1914, 31, No. 8.

² Jour. Amer. Med. Assoc., 1921, 77, 1420; Jour. Infect. Dis., 1918, 22, 211.

³ Arch. f. Kinderh., 1921, 69, 256.

⁴ Med. Rec., 1917, xci, 106.

⁵ Ztschr. f. klin. Med., 1916, lxxxiii, 41.

⁶ Therap. Monatsh., 1918, 32, 12.

Blood was drawn aseptically into sterile bottles, 200 to 300 c.c. being taken from adults and proportionately less from the larger children. No ill effects from bleedings were observed. The sera are separated from the clots, mixed, and preserved with 0.3 per cent. tricresol in bottles of 30 c.c. in a refrigerator. The serum tends to lose in efficiency after keeping more than eight weeks.

The value of the treatment of scarlet fever by means of intramuscular injections of convalescent serum or blood or by intravenous injections of serum is well supported by numerous investigators mentioned above. It is well known that the course of scarlet fever is uncertain, some patients suddenly becoming rapidly better under any plan of treatment. But in severe cases treated *early* with convalescent serum the almost constant fall of temperature and rapid general improvement are striking. It would appear that the treatment is worthy of adoption not only in hospitals for the treatment of scarlet fever, but in private practice as well. In the absence of available convalescent serum normal blood may be used, but the results are not as satisfactory.

Treatment of Scarlet Fever with Normal Serum and Other Non-specific Agents.—Moog¹ and Rehder² have treated scarlet fever with injections of normal serum and the former thought the results were as good as observed with convalescent serum. Holler³ has employed intravenous injections twice daily of 1 c.c. of a 10 per cent. solution of deuterio-albumose, and Ludke⁴ has employed albumose injections in the same manner.

According to these investigations the beneficial effects of serum treatment with normal or immune sera are non-specific and the choice of agent merely a matter of individual preference. The consensus of opinion, however, is to the effect that the results produced by convalescent serum are in a large part specific and that this serum is to be preferred in the treatment of scarlet fever.

TREATMENT OF TYPHOID FEVER

By reason of being the first acute infectious disease treated with vaccines, typhoid fever has since commanded a great deal of attention from the standpoint of treatment with both specific and non-specific vaccines and other biologic agents. Indeed the subject of non-specific protein therapy has been largely developed, clinically at least, by studies of this nature in typhoid fever and arthritis.

Vaccine Treatment of Typhoid Fever.—*Treatment with Plain Vaccine Subcutaneously.*—While killed preparations of the typhoid bacillus have been injected subcutaneously as a method of treatment in typhoid fever since the work of Fränkel⁵ in 1913, the subject is still in the experimental stage. An analysis of the literature upon this subject by Callison,⁶ Watters,⁷ and Krumbhaar and Richardson⁸ show that at best the ordinary type of heat-killed vaccine administered subcutaneously does no harm and may shorten the course of the disease, prevent fever relapses and complications, and yield a slightly lower mortality. The treatment should be given early if at all, while the resistance and general condition of the patient are good. The injections are given subcutaneously and the doses should be modified in individual cases in order to avoid severe reactions. The initial dose may be 100,000,000 bacilli; three days later 250,000,000 or 500,000,000 may be

¹ Therap. Monatsh., 1914, 28, 37.

² Deut. Arch. f. klin. Med., 1916, cxx, 237.

³ Med. Klinik, 1917, 13, 1038.

⁴ Berl. klin. Wchn., 1920, lvii, 344.

⁵ Deutsch. Med. Wchn., 1893, xix, 985.

⁶ Amer. Jour. Med. Sci., 1912, cxliv, 350.

⁷ Med. Rec., 1913, lxxxiv, 518.

⁸ Amer. Jour. Med. Sci., 1915, cxlix, 406.

injected, followed by three or four similar doses at intervals of five days. During convalescence two or more additional doses may be given in an effort to prevent relapses.

Treatment with Sensitized Vaccine Subcutaneously.—Garbat¹ has found the subcutaneous administration of sensitized typhoid bacilli or serobacterin superior to the non-sensitized vaccine. His vaccine was sensitized with the sera of typhoid convalescent cases and usually given subcutaneously in dose of 500,000,000 (250,000,000 to very sick individuals) every five to seven days for three or four doses; at least one injection was given during convalescence to prevent relapse. Seventeen patients were treated, with one fatality. Harmful effects were not observed and the general course of the disease seemed milder and the complications less frequent. Ardin-Delteil² had previously used Besredka's living vaccine, and since then several observers have employed living and dead sensitized vaccines by subcutaneous injection, but the results are quite similar to those observed with plain vaccines administered by subcutaneous injection.

Treatment with Vaccine Intravenously.—In 1913 Thirloix and Bardon³ reported the successful treatment of typhoid fever by the intravenous injection of 2,000,000 to 10,000,000 bacilli. More striking results have been reported by Ichikawa,⁴ Kraus,⁵ and others from intravenous injections of sensitized vaccine. Ichikawa prepared his vaccine by suspending ten loops of fresh typhoid culture in 10 c.c. of human typhoid convalescent serum and incubating for five or six hours. The organisms were then secured by centrifuging, washed three times and suspended in 100 c.c. of saline solution containing 0.3 per cent. tricesol. This vaccine was used unheated in doses of 0.5 c.c. diluted with a syringeful of saline and slowly injected intravenously.

Gay and Chickering⁶ have reported upon the treatment of 53 cases of typhoid fever with intravenous injections of 1/50 to 1/25 milligram of a sensitized, polyvalent, killed typhoid vaccine sediment prepared after the method of Gay and Clappole.

A reaction usually follows these injections. A rigor or chill develops in ten to fifteen minutes accompanied by a rise in temperature of 1 to 3 degrees, reaching its maximum in three hours and then falling. There is also a slight increase of the pulse-rate, slight cyanosis and respiratory distress, a sense of discomfort, and a leukopenia.

The temperature reaches normal or subnormal in about twelve hours accompanied by sweating and amelioration of general symptoms and accompanied by a leukocytosis.

Mild reactions of this kind are desirable and the amounts of sediment required vary with different patients. As a general rule the injections are given every two or three days in slightly increasing amounts until three or four have been given. Gay has not observed any harmful effects and as many as fifteen or sixteen injections have been given a single patient.

The mortality in a series of 98 cases was 6.6 per cent.; in 66 per cent. of the cases a distinct benefit was obtained, as shown by lowered temperature, disappearance or amelioration of subjective symptoms, and an apparently accelerated recovery.

¹ Jour. Amer. Med. Assoc., 1915, 64, 489.

² Compt. rend. Acad. d. sc., 1912, cxi, 1174.

³ Soc. méd. d. Hôp., 1913, 36, 108.

⁴ Ztschr. f. Immunitätsf., orig., 1914, xxiii, 32.

⁵ Wien. klin. Wchn., 1914, xxvii, 1443.

⁶ Archiv. Int. Med., 1916, xvii, 303. (This paper contains a very good review of the literature upon the treatment of typhoid fever with the intravenous administration of sensitized vaccines.)

Comparative Value of Different Vaccines by Subcutaneous and Intravenous Injections.—Gay¹ has recently made a careful survey of the results of treatment with different vaccines administered subcutaneously and intravenously by different observers:

SUMMARY OF RESULTS OBTAINED BY RECENT OBSERVERS (1913-17) IN THE TREATMENT OF TYPHOID FEVER BY VACCINES ADMINISTERED IN VARIOUS WAYS

	OBSERV- ERS.	TOTAL CASES.	ESTIMATES BASED ON.	BENE- FITTED, PER CENT.	MORTAL- ITY, PER CENT.
Untreated vaccine subcutaneously.....	30	1001	512	46	14.5
Sensitized vaccine subcutaneously.....	14	593	239	69	8.0
Untreated vaccine intravenously.....	22	501	233	62	13.0
Sensitized vaccine intravenously.....	12	487	316	85	11.0

These mortality records are within the normal limits of untreated cases, but Gay believes that the results in general were more favorable to the use of sensitized vaccine injected intravenously.

Gay has attributed most of the beneficial results to non-specific effects and principally to the hyperleukocytosis. Apparently the typhoid protein acts much as other non-specific proteins described below; at least the reactions are the same. In addition, however, it is possible that typhoid vaccines in general, including the sensitized sediment, have the property of engendering specific effects in some degree. At least there is generally an increase of agglutinins, and Gay believes that specific antibodies aid in the destruction of typhoid bacilli by the increased numbers of leukocytes.

The Effect of Vaccine Therapy Upon Relapses and Complications in Typhoid Fever.—Vaccine therapy has had little or no influence upon the occurrence of *relapses*. In Gay's series they occurred in 10.2 per cent. which was about the expected number. Gay believes, however, that the administration of a few doses of vaccine by *subcutaneous* injection on alternate days after the temperature has remained normal for twenty-four hours, may aid in preventing relapses; the dose of his vaccine for this purpose is 0.1 milligram corresponding to about 800,000,000 bacilli. This plan has also been recommended by Meyer,² Meyer and Alstaedt,³ and others.

In the treatment of *complications*, as periostitis, glandular suppuration, cholecystitis, and similar complications due to localized infections, a typhoid vaccine may be of considerable aid. The vaccine should be autogenous if possible, and mixed if the lesions are open and other bacteria of probable pathogenic activity are found. Stone⁴ has reported favorably upon the treatment of cases of *typhoid carriers* with the ordinary vaccine, and it would appear that this form of treatment is worthy of trial.

Serum Treatment of Typhoid Fever.—The serum of Chantemesse is the only serum that has been used on a large scale in the treatment of typhoid fever in man.

The serum is derived from horses that have been immunized for several years with bouillon filtrates containing typhoid toxin, chiefly endotoxin, and with typhoid bacilli. Kraus and von Stenitzer, Meyer, Bergell, and Aronson use bouillon filtrates and aqueous bacterial extracts; Besredka

¹ Typhoid Fever, MacMillan Co., 1918, 222.

² Berl. klin. Wchn., 1915, liii, 870.

³ Berl. klin. Wchn., 1915, No. 52, 677.

⁴ Jour. Amer. Med. Assoc., 1910, lv, 1708; Amer. Jour. Med. Sci., 1912, cxliii, 544.

injects dead and then living cultures; MacFadyen uses an endotoxin secured by breaking up cultures frozen at very low temperature. It would appear that a serum should be bacteriolytic and endotoxic, and this probably is best secured by prolonged intravenous immunization of horses with a large number of dead cultures and then with autolysates and living cultures conjointly.

According to Chantemesse, the subcutaneous injection of a few drops of his serum produces leukocytosis and raises the opsonic index of the patient's serum. He emphasizes the fact that the serum should be given early—before the seventh day—and reports that, by its use, the mortality has been reduced from 17 to 4.3 per cent. These results have not been generally confirmed.

Gay and Chickering have treated a few cases with goat immune serum and vaccine, and thought that the beneficial effects were more pronounced than with vaccine alone; further experiences, however, have not confirmed these hopes, although Gay believes that the subject of combined serum and vaccine therapy is one that should be more extensively tested.

Kraus and V. Steritzer¹ and Rodet² had previously used a typhoid immune serum by subcutaneous or intravenous injection, with apparently good results; it is highly probable, however, that the beneficial results were due to non-specific protein shock reactions rather than to specific effects.

Nicolae and Conseil³ have treated 5 patients with the serum of convalescent typhoid patients. The dose was about 130 c.c.; in their experience the duration of the disease was not shortened, but certain nervous symptoms appeared to be improved. Garbat⁴ treated 3 severe cases with subcutaneous and intramuscular injections of convalescent serum; a third case received intravenous injections because the bloods of donors and patient were compatible. Two to three doses of 50 to 100 c.c. of serum were given, with good results, and Garbat believes that this treatment should be considered for the treatment of severe cases when donors are available.

In cases of hemorrhages it would appear advisable to transfuse the whole blood of a convalescent donor or some one who has had typhoid fever, in order not only to furnish immune substances, but to secure a styptic effect as well.

Non-specific Treatment of Typhoid Fever.—From what has been stated above it is apparent that most observers are of the opinion that the beneficial effects to be secured in the treatment of typhoid fever with vaccines and sera and especially by intravenous injection are due to their non-specific effects.

Heterovaccines Intravenously.—Kraus and Mazza⁵ found that the injection of colon vaccine did just as well as typhoid vaccine. Reibmays⁶ and Decastello⁷ employed cholera and colon vaccines, and Stein,⁸ in presenting the results in about 1500 cases of typhoid treated by him both subcutaneously and intravenously, found that by either method of injection colon vaccine gave results similar to typhoid vaccine.

Albumoses Intravenously.—Lüdke,⁹ in 1915, treated 23 cases of typhoid with intravenous injections of deuto-albumose and later 78 cases of typhoid and paratyphoid B infections; no deaths occurred in the latter group and the results were good, especially when the treatment was instituted early in

¹ Deutsch. med. Wchn., 1911, 37.

² Bull. Acad. d. Sci., 1916, 76, 85, 114.

³ Ann. de l'Inst. Pasteur, 1912, 26, 332.

⁴ Jour. Immunology, 1916, 1, 387.

⁵ Deutsch. med. Wchn., 1914, xl, 1556.

⁶ Münch. med. Wchn., 1915, lxii, 610.

⁷ Mitt. d. Gesellsch. f. inn. Med., 1915, 14, 105.

⁸ Wien. klin. Wchn., 1919, 32, 895.

⁹ Münch. med. Wchn., 1915, lxii, 321.

the disease. He injected 0.5 to 1.5 c.c. of a 10 per cent. solution every two or three days as indicated.

Holler¹ treated 350 cases of typhoid fever with injections of deuterio-albumoses, giving two to four daily injections of 10 per cent. solutions beginning with 0.5 c.c. and increasing as indicated. The average duration of the disease was ten days and the mortality $\frac{1}{2}$ per cent.

Galambos² treated 25 cases with injections of 1 c.c. of a 4 per cent. solution of deuterio-albumose and obtained a critical drop in the temperature in 50 per cent. of the cases. These treatments appeared to be more effective than the injection of heterovaccines of staphylococci, gonococci, and colon bacilli. Galambos has also employed injections of physiologic saline solution (100 c.c. intravenously) and methylene-blue by mouth in dose of 1.2 gm. per day divided into four-hour doses. The intravenous injection of saline was found too bothersome for routine use; methylene-blue was found to be a stimulant and the euphoria so commonly observed in non-specific therapy was apparent in most instances, but protein-split products appeared to yield the best results. Medication was continued some days after the temperature reached normal.

In regard to dosage of proteoses, one of two plans may be followed: either to give sufficient to provoke a general reaction and perhaps repeat the dose after several days if the fever has not been altered, or to give small daily doses as employed by Holler, Jobling, and Petersen.

Milk Intramuscularly.—Owing to the greater ease of administration, intramuscular injections of 5 to 10 c.c. of milk boiled for ten minutes and cooled, have become quite popular among non-specific agents in the treatment of typhoid and paratyphoid fevers. Decided advantages are the styptic effects of milk and the greater ease in controlling the reactions by attention to dosage, in addition to the greater ease of administration. Schmidt,³ Saxe,⁴ Müller,⁵ Cormaldesi,⁶ Grote,⁷ and others have rendered favorable reports, and especially when the injections were started early. In most cases a rise of temperature persisting for about two days was followed by lysis to a normal level. There was only rarely chills and the reactions were generally mild.

Karell and Luksch⁸ have also employed milk injections for the treatment of *bacillus carriers* with apparently good results.

Mechanism of Action of Non-specific Agents in the Treatment of Typhoid Fever.—A great deal of attention has been given the subject of antibody production and particularly of agglutinins, under stimulation by non-specific agents. Some investigators have reported an increased production of agglutinins, and a few an increase of bacteriolysins and opsonins; others have reported negative results. Very probably the injection of non-specific agents does tend to stimulate the antibody producing tissues and particularly after these have become sensitized.

Doubtless the leukocytosis is an important factor in overcoming the infection, and especially when aided by the increased production of antibodies, as suggested by Gay. Lüdke and Petersen also suggest that after the reactions with alterations in cellular permeability and stimulation, the cells acquire an increased resistance to the intoxication.

Precautions in the Treatment of Typhoid Fever with Vaccines and Non-specific Agents.—There are two chief dangers according to Petersen to keep

¹ Med. Klin., 1917, 13, 1038.

² Wien. klin. Wchn., 1916, 29, 1041.

³ Med. Klin., 1916, 12, 171.

⁴ Wien. klin. Wchn., 1916, 29, 1043.

⁵ Deutsch. med. Wchn., 1918, xlv, 545.

⁶ Riforma med., 1920, 36, 296.

⁷ Münch. med. Wchn., 1919, lxvi, 307.

⁸ Wien. klin. Wchn., 1916, 29, 187.

in mind, and especially when typhoid or non-specific vaccines and protein-split products are injected intravenously:

(a) *Overintoxication*.—With a profoundly toxic patient we must be reasonably certain that he is able to bear the increase in intoxication which is probably due to a rapid destruction of typhoid bacilli and poisons from the disease foci.

(b) *The possibility of hemorrhages and perforation* due to increased peristalsis and increased congestion of the intestinal tract.

Petersen states (italics are mine): "*If the pulse is over 100 and there are evidences of vasomotor instability, if the patient is profoundly toxic or cachectic, if there has been any bleeding—epistaxis, gastro-intestinal, etc.—if cyanosis is present, if the disease has continued for several weeks before treatment is commenced, or if there is any evidence of pneumonic complications, it is not advisable to try non-specific therapy. If given under such conditions the clinician must consider the dangers involved and seriously weigh the chances for collapse or hemorrhage or perforation, and determine whether or not they are overbalanced by possible advantages.*"

TREATMENT OF INFLUENZA

The bacteriology, immunity, and vaccine prophylaxis of influenza have been discussed in Chapter XXXV. From the standpoint of mortality and biologic therapy most interest in the last pandemic was commanded by the complicating bronchopneumonia. The biologic therapy of influenza itself, however, is to be briefly considered, the treatment of bronchopneumonia being discussed later.

Serum Treatment of Influenza.—Ordinarily influenza is of such short duration and mild character that serum therapy is not to be considered. Anti-influenzal serum is available, but its use is confined to the treatment of influenzal meningitis. During the last pandemic the intravenous injection of 20 to 50 c.c. of convalescent human serum was being employed, but especially in the treatment of the bronchopneumonia; I shall refer to this treatment in more detail under that subject.

Huff¹ has mentioned the beneficial effects following the subcutaneous injection of 8 c.c. of *human convalescent influenza serum* in the treatment of influenza of a child. Meille² reports favorable results from the subcutaneous injection of 1 or 2 c.c. of the patient's own serum (autoserum).

A number of clinicians, as Kautsky,³ Bettinger,⁴ Lustig,⁵ and Crohn⁶ have employed subcutaneous and intramuscular injections of *diphtheria antitoxin* in doses of 1 to 10 c.c. and claim good results in uncomplicated influenza and cases with pneumonia. The effects were evidently non-specific and doubtless would have been observed to the same degree if normal horse-serum had been employed.

Vaccine Treatment of Influenza.—The usual stock mixed vaccine of influenza bacilli, streptococci, pneumococci, and staphylococci (see under Vaccine Prophylaxis of Influenza) has been employed by some physicians for the routine treatment of influenza. When administered early in the disease it is believed to reduce the incidence of bronchopneumonia and bronchitis and to shorten the course of the general infection.

Borden and Leopold⁷ observed that vaccine treatment of 60 cases of

¹ Brit. Med. Jour., May 10, 1919, 575.

² Policlinico, 1918, 25, 1055.

³ Med. Klin., 1919, 15, 69.

⁴ Münch. med. Wchn., 1919, 66, 125.

⁵ Med. Klin., 1919, 15, 42.

⁶ Münch. med. Wchn., 1920, 67, 1521.

⁷ U. S. Naval Med. Bull., 1919, 13, No. 4.

influenza had little immediate effect on the course of the disease, except a fall of temperature probably twenty-four hours earlier than control cases; 5 per cent. of these cases developed pneumonia, while of 221 control cases 35.3 per cent. developed pneumonia. Wynn has treated 107 cases with subcutaneous injections of mixed vaccine, the dose being 80,000,000 to 100,000,000 of each organism. He states that in simple uncomplicated influenza a single injection may abort the disease. The following table by Wynn, emphasizes the importance of early treatment, not only upon the duration of the disease, but upon the mortality as well:

DAY OF INJECTION.	NUMBER.	RECOVERED.	DIED.	TEMPERATURE NORMAL IN	
				Twenty-four hours, per cent.	Forty-eight hours, per cent.
First.....	28	28	.	71.4	85.7
Second.....	23	22	1	47.8	56.5
Third.....	22	20	2	50	72.7
Fourth.....	20	15	5	30	40
Fifth.....	14	12	2	35.7	63.5
Total.....	107	97	10	50	65

TREATMENT OF PNEUMOCOCCUS LOBAR PNEUMONIA

Acute lobar pneumonia, with its clear-cut clinical course, unsatisfactory and difficult treatment, uncertain prognosis, and high mortality, was one of the diseases in which the earliest efforts were directed toward discovering a specific serum therapy. Since the pioneer work of the Klemperers in 1891, numerous investigators have prepared serums that have yielded either indifferent results or proved beneficial in but a limited number of cases, so that there has been no well-established form of specific therapy.

Recent investigations by Neufeld and Händel¹ in Germany, and by Dochez,² Cole,³ and Gillespie,⁴ in the Rockefeller Institute, have disclosed several reasons for the failure of serum therapy in pneumonia, and have emphasized the importance of the following factors:

1. The serum should correspond to the type of pneumococcus causing the infection.

2. The serum must be administered in large doses, and preferably intravenously.

3. To be most effective the treatment should be given as early as possible.

The investigators in the Rockefeller Institute have divided the pneumococci causing lobar pneumonia into four main groups, and have worked out a method for the rapid identification and classification of the particular pneumococcus that is the etiologic factor in a given infection, so that in Type I infections the proper administration of the corresponding immune serum has yielded encouraging results in the serum treatment of Type I pneumonia. These researches are of importance not only in this connection but also from the fact that they may have disclosed the reasons for failure

¹ Ztschr. f. Immunitätsf., orig., 1909, 3, 159; Arb. a. d. k. Gsndhtsamte; 1910, xxiv, 169; *ibid.*, 1910, xxxiv, 293; Berl. klin. Wchn., 1912, xlix, 680.

² Jour. Exper. Med., 1912, xvi, 665, 680, 693; Jour. Amer. Med. Assoc., 1913, lxi, 727.

³ Jour. Exper. Med., 1912, xvi, 644; Arch. Int. Med., 1914, xiv, 56.

⁴ Jour. Amer. Med. Assoc., 1913, lxi, 727; Jour. Exper. Med., 1914, xix, 28.

in the treatment of streptococcus and other infections, and that similar studies in these conditions may insure for serum therapy a definite and valuable rôle in the treatment of disease.

The Nature of Lobar Pneumonia.—The frequency with which the *Diplococcus pneumoniae* is found in the local lesion and in severe cases in the blood-stream of pneumonia patients, and the more recent experimental studies of Wadsworth,¹ Meltzer,² Wollstein and Meltzer,³ Winternitz, Kline and Hirschfelder,⁴ leave little doubt regarding the etiologic relationship of this micro-organism to lobar pneumonia. Much still remains to be learned, however, regarding the method of infection and the nature of the resulting disease. While pneumococci are to be found living in the upper air-passages as harmless parasites, it is probable that those causing infection differ inherently as regards adaptation or virulence for man. In addition it is likely that general resistance is lowered in some more or less peculiar manner, and experimental studies in animals, as well as the course of the disease in man, suggest most strongly that local changes in the respiratory tract may precede the infection, so that a combination of factors, such as the virulence of the organisms and the diminished general and local resistance, play a part in the production of lobar pneumonia.

The investigations of Blake and Cecil,⁵ previously referred to in Chapter XXXV, have been very instructive and indicate that pneumococci probably penetrate the tissues at or near the root of the lung and reach the pulmonary tissues along the framework and lymphatics with the production of pneumonitis. According to this view, lobar pneumonia is a bronchogenic infection, a direct infection, rather than a primary general pneumococcus bacteremia, with subsequent selective localization in the lungs.

While pneumococci may be found in the blood of the most severe cases, the general symptoms are apparently due to intoxication with a poison or toxin derived primarily from the pneumococci, and secondarily from the exudate in the local lesions. The studies of Rowntree,⁶ Medigreceanu,⁷ and Peabody,⁸ showing chlorin retention; of Peabody,⁹ showing progressive loss in the oxygen-combining power of the hemoglobin, due to the formation of methemoglobin; of Medigreceanu,¹⁰ showing a deficiency of oxydase or lessened power of the tissues to carry on proper oxidation, and of Neufeld and Dold,¹¹ Rosenow,¹² Cole,¹³ Wadsworth,¹⁴ Jobling and Strouse,¹⁵ Weiss and the writer¹⁶ indicating the presence of exotoxins and endotoxins within the pneumococci—all these support the view that in pneumonia there is well-marked intoxication, and this, in addition to the effects of the local pulmonary consolidation on the heart, respiration, and nervous system, constitute the main features of the infection.

Regarding the mechanism of recovery from pneumonia, there is little

¹ Amer. Jour. Med. Sci., 1904, cxxvii, 851.

² Jour. Exper. Med., 1912, xv, 133.

³ Jour. Exper. Med., 1913, xvii, 353; *ibid.*, 1913, xviii, 548.

⁴ Jour. Exper. Med., 1912, xvii, 657; *ibid.*, 1913, xviii, 50.

⁵ Jour. Exper. Med., 1920, 31, 403.

⁶ Bull. Johns Hopkins Hosp., 1908, xix, 367.

⁷ Jour. Exper. Med., 1911, xiv, 289.

⁸ Jour. Exper. Med., 1913, xvii, 71.

⁹ Jour. Exper. Med., 1913, xviii, 7.

¹⁰ Jour. Exper. Med., 1914, xix, 309.

¹¹ Berl. klin. Wchn., 1911, xlviii, 1069.

¹² Jour. Infect. Dis., 1911, ix, 190.

¹³ Jour. Exper. Med., 1912, xvi, 644; *ibid.*, 1914, 20, 346.

¹⁴ Jour. Exper. Med., 1912, 16, 78.

¹⁵ Jour. Exper. Med., 1913, xviii, 597.

¹⁶ Jour. Immunology, 1918, 3, 395.

definite information. The recent studies of Neufeld, Dochez, and Clough indicate that antibodies are produced at or about the time of the crisis, and that these are probably responsible for the destruction of the bacteria in the circulating blood, and, to a greater extent, in the local lesion. In the resolution of the local lesion it is probable that ferments play an important part. That resolution does not occur earlier may be due to the overbalancing of the leukocytic ferments by the antiferments of the serum, and the lytic ferments become active only when they reach a point of excess over the antiferments, causing a solution of the fibrin, relieving tension, and affording an outlet for the exudate. According to Vaughan, the pneumococci may be considered as furnishing a ferment that brings about the production of a specific antiferment, capable of reacting upon its substratum, the ferment and the new bacterial tissue, and causing its destruction by a process of solution. Pneumococci in the resolving lesion are probably destroyed by leukocidins released through disintegration of leukocytes, by fatty acids, and probably by antibacterial substances in the blood.

While it is true that immunity does not usually follow an attack of pneumonia, and, indeed, the patient is apparently hypersusceptible, it has been found experimentally that the antibodies are highly specific for the particular organism causing an infection. Reinfection is, therefore, possible with an organism belonging to another group, and liability to reinfection may be increased because of lowered local and general resistance due to the previous attack.

Thomas¹ has recently reported a recurrent attack of Type I pneumonia in the same individual within a month after a previous attack with treatment by Type I serum; this indicates that an active immunity, if produced at all, was of brief duration, and that passive immunity conferred by the immune serum was less than a month in duration.

SERUM THERAPY OF PNEUMOCOCCUS PNEUMONIA

Indications.—The indications for specific serum therapy are mainly twofold: first, to destroy any pneumococci present in the blood and in the local lesion; or, if the latter is impossible because of mechanical obstacles that interfere with the circulation and prevent access of the antibodies to the cocci, to at least prevent extension of the lesion by preventing the multiplication of organisms at its margin; second, to neutralize the toxins produced during the course of the disease.

It would, of course, be highly desirable to have at our command a serum that would cause solution of the local exudate and bring about a crisis and a cure. It is hardly reasonable to expect, however, that a serum can be produced that will contain digestants for fibrin and leukocytes. The local lesion is most likely to be harmful because of the toxic substances that emanate from it, and not because so large an area of lung is temporarily incapacitated and the heart embarrassed. A serum that will prevent general bacteremia, limit the extension of the local lesion, and neutralize the toxins while nature is preparing to react upon the exudate with a ferment, is probably fulfilling all that may be expected of a specific serum therapy.

Types of Pneumococci.—Neufeld and Händel have shown that an immune serum produced by the injection of a given variety of pneumococci into an animal was not effective against all forms of pneumococci. In the Rockefeller Hospital a serum, known as Serum 1, prepared by immunizing a horse with a culture obtained from Neufeld, was found to protect against only about one-half the types of pneumococci (Group I) studied. By immunizing

¹ Amer. Jour. Med. Sci., 1920, 161, 103.

rabbits to each of the types that were not acted upon by Serum 1, and testing the immune serum against all strains by cross-agglutination and by cross-protection experiments, it was found that a number of the serums possessed the same properties, thus indicating that their respective cultures belonged to the same general group (Group II). By immunizing a horse with one of these, Serum 2 was produced. In Group III are placed all the organisms of the so-called *Pneumococcus mucosus* type. In Group IV are included all the varieties of pneumococci against which Serums 1 and 2 are not effective, and which, from their other properties, do not belong in Group III. Animals may readily be immunized to any member of this Group IV, and the serum of the immunized animal is protective against the strain used for immunization, but in no instance has this serum been found effective against any other member of this group or against the organisms of the other groups. While no cultural or morphologic differences between the members of Group I, II, and IV exist, it has been found possible to group them by the agglutination reaction in exactly the same manner as by protection experiments.

Determining the Type of Pneumococcus.—The method is described on page 286.

Preparation of Antipneumococcus Serum.—The method commonly employed is that developed by Cole and his associates¹ in the Rockefeller Institute. Horses are immunized with mixed cultures of pneumococci belonging to Types I, II, III, and IV or of Type I alone; the Hygienic Laboratory requires a minimum standard of protective value against only virulent Type I pneumococci.

Sound and fairly heavy horses are chosen proved free of glanders.

Broth cultures of the pneumococcus should be of such virulence that 0.000001 c.c. kills mice regularly. Cultures are grown in beef peptone broth +0.3 to 0.5 for twelve to fifteen hours and should show 200,000,000 to 300,000,000 bacteria per cubic centimeter. About 350 c.c. of this broth is thoroughly centrifuged and the cocci suspended in 125 c.c. of sterile saline solution, heated in a water-bath at 56° C. for thirty minutes and kept on ice.

Twenty c.c. of this suspension are injected intravenously every day for six days, followed by a resting interval of seven days.

A fresh culture is now prepared in the same manner and a second series of six daily injections given.

Seven days later three daily intravenous injections of living pneumococci are given, the first dose being a suspension of cocci removed from 20 c.c. of the original broth culture, the second dose being the cocci from 40 c.c., and the third, the cocci from 80 c.c. of broth. If the febrile reactions are severe, smaller doses are given.

Seven days later the series is repeated giving the cocci from 100, 150, and 200 c.c. of broth on the three days respectively. Seven days later a third series of injections are given comprised of the cocci from 100, 200, and 300 or 400 c.c. of broth on the three days respectively.

About seven days later a small amount of blood is drawn and the serum tested. If satisfactory a large bleeding may be made followed by a rest of three or four days, when a fourth series of intravenous injections of cocci from 50, 80, and 100 c.c. of broth are given on the three days respectively. After a week the serum is again tested, and if of standard strength, bleeding may be done on the tenth day. If the serum is not sufficiently strong, a second series of live cultures is given, again keeping the dosage fairly small, never giving more than the bacteria from 300 to 400 c.c. of broth and always bleeding about ten days after the last injection.

After large bleedings which are conducted in an aseptic manner, the serum is tested for sterility by cultural and animal tests and preserved with 5 c.c. of chloroform per liter.

In view of the large doses of serum required in the treatment of lobar pneumonia and the possible injurious effects of the large amount of horse-serum protein injected, Gay and Chickering² and Chickering³ have endeavored to concentrate the immune serum by adding relatively small amounts of the extract of pneumococcus and recovering the resulting precipitate. These precipitates have been found to contain practically all the protective antibodies of the original serum and relatively small amounts of protein as compared with the original serum. At the present time, however, this method is not being employed and whole serum is generally administered.

Standardization of Antipneumococcus Serum.—At the present time the protection test alone is being employed. The following method, modified after that devised by Neufeld,⁴ is employed in the Rockefeller Institute and the majority of laboratories concerned in the preparation of this serum:

White mice weighing 18 to 22 gm. are employed. The culture of Type I pneumococcus

¹ Monograph No. 7 Rockefeller Institute.

² Jour. Exper. Med., 1915, xxi, 389.

³ Jour. Exper. Med., 1915, xxii, 248.

⁴ Ztschr. f. Immunitätsf., orig., 1909, 3, 159; Arb. a. d. k. Gsndhtsamte., 1910, 34, 166; *ibid.*, 1910, 34, 293; Berl. klin. Wchn., 1912, 39, 680.

should be of such virulence that 0.000001 c.c. of an eighteen-hour broth culture injected intraperitoneally kills a mouse in forty-eight hours.

Various dilutions of the culture are made with peptone broth, using a fresh pipet for each, in such manner that 0.5 c.c. carries 0.2, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 c.c. of the original broth culture.

For the test 2 c.c. of serum are diluted with 3 c.c. of broth so that 0.5 c.c. carries 0.2 c.c. undiluted serum.

When injecting the animals, 1 c.c. of diluted serum and 1 c.c. of the diluted culture are taken up into a 2 c.c. Luer or Record syringe, mixed, and 1 c.c. injected intraperitoneally into each of two mice. It is well to start with the weakest culture first, working up to the largest amount, separate syringes being used for each, or the one syringe washed out with hot sterile water and cooled between the injections.

Three control mice receive injections of 0.00001, 0.000001, and 0.0000001 c.c. of the culture alone.

The mice are properly marked and observed for at least forty-eight hours.

To be satisfactory a serum in dose of 0.2 c.c. should protect against at least 0.1 c.c. of the culture of the above-mentioned virulence, that is, against about 100,000 fatal doses of culture.

Action of Antipneumococcus Serum.—The curative and protective value of this serum depends mainly upon bacteriolysins, bacteriotropins, and antitoxins. The first are readily demonstrated in protection tests and also in pneumonic patients when pneumococci in the blood-stream are destroyed. Bacteriotropins may likewise be demonstrated experimentally and in the blood of patients if the corresponding organism is used in the tests (Neufeld, Strouse). The antitoxic properties are shown clinically and also *in vitro* by neutralization of the hemotoxic poison obtained by dissolving pneumococci in bile. As shown recently by Bull,¹ the agglutinins in the serum may play a very active rôle by producing an agglutination of the cocci in the blood-stream followed by phagocytosis.

According to Avery² the antibodies are associated or combined with that fraction of the globulins precipitated by 38 to 42 per cent. saturation with ammonium sulphate.

Types of Pneumococci in Relation to Serum Treatment and Mortality of Pneumonia.—The value of serum treatment has been established for Type I pneumonias, is doubtful in Type II, and is without beneficial effects in Type III and Type IV infections.

The incidence of these types of pneumococci in pneumonia is indicated by the investigations of Cole and Longcope in New York, and Richardson in Philadelphia; since the influenza epidemic the Type IV strains have been more abundant than the combined fixed Types I, II, and III.

INCIDENCE OF TYPES OF PNEUMOCOCCI IN LOBAR PNEUMONIA

	COLE. Per cent.	LONGCOPE. Per cent.	RICHARDSON. Per cent.
Type I.....	33	23	31
Type II.....	32	21	20
Type III.....	9	14	6
Type IV.....	20	40	43
Other bacteria, 6 per cent.			

MORTALITY IN LOBAR PNEUMONIA (NO SERUM)

	COLE. Per cent.	LONGCOPE. Per cent.	RICHARDSON. Per cent.
Type I.....	25	12.5	30
Type II.....	29	72.7	25
Type III.....	45	85.7	50
Type IV.....	12.5	23.8	12

¹ Jour. Exper. Med., 1915, 22, 466.

² Jour. Exper. Med., 1915, 21, 133.

Type I pneumonias, therefore, constitute about 30 per cent. The mortality, however, has varied greatly at different times in different parts of the country, but in general terms ranges from 15 to 30 per cent.

Cole states that serum should be given only to Type I cases; this requires, of course, a delay of twelve to twenty-four hours or longer for the necessary typing. Park has always stated that in severe cases when a delay in typing is unavoidable, that a dose of Type I or polyvalent serum should be given as soon as possible and the later injections only in case the infection proves to be Type I infection. In routine practice this means the administration of serum to some cases other than Type I infections, but appears justifiable in the treatment of severe infections when facilities are poor for the necessary preliminary typing.

Polyvalent sera prepared by biologic laboratories doubtless contain small amounts of antibodies for Types II and III in addition for Type I, but it is doubtful that they are of benefit in the treatment of any but Type I pneumonias, except for the possible beneficial results from non-specific effects.

Administration of Antipneumococcus Serum.—To obtain the best results *the serum should be given as early as possible and intravenously*. In young children, when the giving of an intravenous injection is quite difficult or impossible, the muscles of the buttocks should be substituted. Certainly small doses given subcutaneously are almost devoid of effect. The procedure in use in the Rockefeller Institute¹ consists in injecting 0.5 c.c. of serum subcutaneously to discover if hypersensitiveness exists and to produce anti-anaphylaxis. About one hour later from 90 to 100 c.c. of the serum, diluted one-half with salt solution, are injected intravenously. The condition of the patient serves as a guide in the later treatment.

Cole² has recently stated that "this dose should be repeated every eight hours until the fall of temperature and amelioration of symptoms indicate that the infection has been overcome. The serum injected should be at body temperature, and it should be injected very slowly. The total amount required in the average case is from 200 to 300 c.c., though in severe cases, treated late in the disease, it may be necessary to employ much larger amounts, even as much as 1000 c.c."

It has been shown experimentally that, in the presence of a maximum degree of infection, no amount of serum, however large, is effective. This suggests that the body must furnish a second substance to act with the antibodies in the serum, and indicates the early administration of serum before the infection has reached too extreme a grade. I would also suggest that the body may be deficient in bacteriolytic complements, and that the effect of a serum may be enhanced by adding *fresh sterile human serum*—say 5 c.c. to each 100 c.c. of immune serum—just prior to administration.

Meyer³ has recently shown that the opsonins in antipneumococcus serum may be reactivated by the addition of small amounts of fresh human serum.

Results in the Serum Treatment of Pneumonia.—It is hardly necessary to review the numerous reports that have been made in past years, because in most instances the serum was administered subcutaneously and in too small doses to be of value, even granting that it contained antibodies for the particular infection. Prior to the investigations of recent years showing the serologic grouping of pneumococci, horses were immunized with a

¹ Avery, Chickering, Cole and Dochez, Monograph No. 7, Rockefeller Inst., 1917.

² Jour. Amer. Med. Assoc., 1921, 76, 111.

³ Jour. Infect. Dis., 1920, 27, 82.

variety of strains and the sera were polyvalent. Furthermore, the sera were not very potent and in view of the administration of small amounts, the results were not encouraging.

At the present time the serum treatment of pneumonia is practically confined to the Type I infections. Richardson,¹ in an excellent investigation and review of the literature, has shown that roughly about one-fourth of all cases of lobar pneumonia are due to Type I pneumococci. The mortality of this group treated without serum is from 25 to 30 per cent. or higher. Cole reports that "among 195 cases treated with serum in the Hospital of the Rockefeller Institute, only 18 deaths have occurred, a case mortality rate of but 9.2 per cent. Reports of 300 additional cases treated with serum have been collected from the literature, making a total of 495 cases. The case mortality rate in these 495 cases has been 10.5 per cent."

Cecil and Blake² have shown the specific curative effects of Type I serum in the treatment of severe experimental Type I pneumonias of monkeys. All treated animals recovered; all untreated controls died. Frequent injections were found important, and when serum treatments were instituted late in the disease, the injections were required over a longer period of time. Normal horse-serum was observed to be without beneficial effects.

Thomas³ has recently reported upon the serum treatment of 50 Type I cases in New York City. The course of the disease appeared to have been shortened in 4 cases; in 8 cases the improvement appeared to have been but transitory, while in the remaining 38 the duration and outcome of the disease do not appear to have been demonstrably affected by the serum.

Aside from an apparent influence upon mortality the general effects of the serum are sometimes good. The blood of the patient becomes sterile following the injection of serum. The progression of the local lesion in the lung is usually arrested. The subjective and objective symptoms of the disease are lessened. Following the injection of serum most patients seem to feel better, and in a number of them there is an apparent lessening in the degree of intoxication. While in no case is one injection sufficient to bring about a crisis, in all except the fatal cases the serum has apparently an ultimate favorable effect, lowering the temperature and shortening the course of the disease.

The sum total of experience indicates, however, that the serum treatment of lobar pneumonia is not as promising as the earlier reports indicated; that it should be confined to Type I pneumonias and that the serum must be given early, in large amounts and by intravenous injection.

The procedure is not wholly without danger. Thomas states that 2 deaths from anaphylaxis are reported in the literature. It is necessary to give the serum with considerable caution. The skin tests for anaphylaxis and the possible reactions, anaphylactic, so-called thermal and serum disease, have been considered in preceding chapters.

Kyes' Antipneumococcus Serum.—Kyes⁴ has described a method of preparing antipneumococcus serum by the immunization of chickens with massive intraperitoneal doses of virulent pneumococci. As is well known chickens possess a high degree of natural immunity to pneumococci, and both Kyes and more recently Bull and McKee,⁵ have found that normal chicken serum is capable of protecting mice and guinea-pigs against infection. Bull and McKee found that protective substances were present for

¹ Jour. Lab. and Clin. Med., 1919, 4, 484.

² Jour. Exper. Med., 1920, 32, 1.

³ Jour. Amer. Med. Assoc., 1921, 77, 2101.

⁴ Jour. Amer. Med. Assoc., 1911, 56, 1878.

⁵ Amer. Jour. Hyg., 1921, 1, 284.

each serologic type of pneumococcus and that they may be selectively removed from the serum by adsorption.

Kyes¹ administers this serum intravenously in doses of 2.5 c.c. irrespective of the type of pneumonia. Usually one injection is given daily until the temperature remains below 100° F. Sometimes two injections are given per day during the first few days of especially sick individuals. The average number of injections is three.

Of 538 cases not treated with serum the mortality was 45.3 per cent. Of 115 cases under identical conditions treated with his serum, the mortality was 20.8 per cent. or a reduction of more than one-half.

Gray² has reported upon the use of this serum in the treatment of lobar pneumonia in Camp Grant. The doses were increased from 2.5 c.c. once or twice daily to 5 to 10 c.c. twice daily. In a few cases as much as 30 c.c. was given daily. Commonly a total of 60 to 90 c.c. was given in cases that recovered.

In one group of 322 cases of typical lobar pneumonia the mortality was 7.7 per cent. A second group of 234 cases of epidemic pneumococcus-bronchopneumonia the mortality was 16.7 per cent. as compared with 53.6 per cent. of 1684 control cases. In a third group of 118 cases of a typical pneumococcus pneumonia of lesser virulence, the mortality was 4.3 per cent.

Treatment of Pneumococcus Pneumonia with Huntoon's Antibody Solution.—Huntoon and his co-workers³ have recently described a solution of antibodies for pneumococci Types I, II and III combined, secured from polyvalent antipneumococcus serum by dissociating antigen-antibody complexes by various means (for a more complete description, see page 150). The resulting solution of antibodies is remarkable because it yields negative or poorly defined reactions for proteins, does not regularly sensitize guinea-pigs, and proves protective for mice inoculated with virulent pneumococci. From the experimental standpoint it would appear worthy of trial in the treatment of lobar pneumonia, but in the experience of Cecil the intravenous injection sometimes elicits very severe reactions. Recently Cecil and Larsen⁴ have reported the following results in the treatment of pneumonia with this polyvalent antibody solution:

(a) In 424 cases of pneumococcus pneumonia treated with pneumococcus antibody solution, the death-rate was 21.4 per cent. A control group of 410 cases in the same institution showed a death-rate of 28.3 per cent.

(b) Pneumococcus antibody solution produces its most striking effect in pneumococcus Type I pneumonia. In a series of 156 treated cases the death-rate was 13.3 per cent.; while a control series of 162 cases showed a death-rate of 22.2 per cent. A definite but less marked effect was observed in cases of pneumococcus Types II and IV pneumonia which were treated with antibody solution. The antibody solution had no effect whatever on the death-rate in pneumococcus Type III pneumonia.

(c) The death-rate of streptococcus pneumonia was not favorably influenced by antibody solution treatment.

(d) In the series of patients with pneumococcus pneumonia treated with antibody solution, 28.8 per cent. recovered on or before the fifth day. In the control series, only 7.9 per cent. recovered on or before the fifth day.

(e) There were forty-four severe complications in the series of 424

¹ Jour. Med. Research, 1918, 38, 495.

² Amer. Jour. Med. Sci., 1920, 159, 885.

³ Jour. Immunology, 1921, 6, 117, 123, 185.

⁴ Jour. Amer. Med. Assoc., 1922, 79, 343.

pneumococcus pneumonias treated with antibody solution, while the control series of 410 pneumococcus pneumonias showed fifty-four severe complications.

The solution may be given subcutaneously or intravenously—the latter has yielded the best results, but is likewise more likely to produce severe reactions.

The initial dose by subcutaneous injection should be at least twice the intravenous dose or about 50 c.c. and by intravenous injection from 25 to 50 c.c. for adults. The usual procedure is to administer doses at twenty-four-hour intervals as long as the temperature remains above 100° F. In cases where the temperature has not dropped promptly, a second dose should be given within the first twenty-four hours.

Subcutaneous injection produces a local reaction; intravenous injection almost always produces a chill and sharp rise of temperature in from thirty to forty minutes, the reaction being similar to the "foreign protein reaction." Severe reactions of this sort have generally occurred after the first injection given on the fifth or later days of the disease.

Best results have followed the *early* administration of the solution, that is, as soon as the diagnosis has been made. Typing of the pneumococcus is not required except as a matter of interest and record. Cases first treated on or after the fifth day should be injected subcutaneously rather than intravenously. In children twelve years or less of age this treatment may be omitted unless the disease is particularly acute. Further experience is required before a final opinion may be expressed of the practical therapeutic value of this solution.

VACCINE TREATMENT OF PNEUMOCOCCUS PNEUMONIA

Vaccines by Subcutaneous Injection.—While pneumococcus vaccine has been employed by a large number of physicians in the treatment of lobar pneumonia, the curative value of this procedure is still doubtful. Stoner,¹ Allen,² Morgan,³ Harris,⁴ Lyons,⁵ Wynne,⁶ and others have reported favorably upon the vaccine treatment of pneumonia as based upon mortality statistics and clinical impressions; Charteris,⁷ on the other hand, failed to observe beneficial results. Rosenow and Hektoen⁸ have prepared a vaccine of partially autolyzed pneumococci which they believe proved of value in the treatment of pneumonia.

Recently Rosenow⁹ has described the preparation and administration of this vaccine and believes that it favorably influences the mortality and complications of pneumonia. The vaccine is prepared by growing virulent strains of pneumococci of the different types in glucose broth from eighteen to twenty-four hours, centrifugalizing, and suspending the sediment in salt solution so that 1 c.c. contains about 15,000,000,000 pneumococci. The suspension is shaken with ether and then incubated at 37° C. until autolysis has occurred, that is, until 95 per cent. of the organisms have become Gram-negative, and 5 c.c. of the suspension produce few or no toxic symptoms in guinea-pigs. The dose of this vaccine for adults is 1 c.c. administered

¹ Amer. Jour. Med. Sci., 1911, cxli, 186.

² Vaccine Therapy and Opsonic Treatment, Blakiston, Philadelphia, 1913.

³ Proc. Roy. Soc. Med., 1910, iii, Suppl. 65.

⁴ Brit. Med. Jour., 1909, 1, 1530.

⁵ Brit. Med. Jour., 1913, 1, 992.

⁶ Brit. Med. Jour., 1915, 1, 458.

⁷ Glasgow Med. Jour., 1912, lxxvii, 19.

⁸ Jour. Amer. Med. Assoc., 1913, lxi, 2203.

⁹ Jour. Amer. Med. Assoc., 1918, 70, 759.

subcutaneously every day until the temperature reaches normal. Putnam¹ has reported favorably upon the use of this vaccine. Cecil,² however, believes that great caution is to be observed in recommending the treatment of pneumonia by vaccines of whatever modification; and that it is difficult to evaluate the results of vaccine therapy, reported in this disease, because so many factors may possibly determine the question of death or recovery.

If vaccine therapy is employed it would appear necessary to administer it as early as possible. For this reason stock polyvalent vaccines are required. The dose and administration of the Rosenow-Hektoen vaccine is given above. Ordinary saline vaccines may be prepared in such manner that each cubic centimeter contains 250,000,000 of each of the four types; the first dose may be 0.1 c.c. by subcutaneous injection followed by daily or every other day injections in gradually increasing amounts until two to four doses have been given. Cohen,³ who is an ardent believer in the use of vaccines as part of the treatment of lobar pneumonia, employs a mixed stock vaccine of pneumococci Types I, II, and III, streptococci, staphylococci, and *M. catarrhalis*, containing a total of 800,000,000 per cubic centimeter. The first dose is about 0.2 c.c. (125,000,000) before the case is typed. Three days later 0.4 c.c. (250,000,000) are given and repeated at three-day intervals in gradually increasing amounts. The usual case receives two or, at most, three doses, the injections being stopped when resolution of the pneumonia takes place.

In *delayed resolution* vaccine therapy may prove of distinct aid. It is my custom to prepare autogenous vaccines of pneumococci, streptococci, and staphylococci from the sputum, using equal proportions of each organism in such manner that each cubic centimeter contains a total of 2,000,000,000. The first dose is 0.1 c.c. and subsequent injections are given at three- to five-day intervals in gradually increasing amounts.

Vaccines by Intravenous Injection.—Rosenow and Falls⁴ have injected vaccines of autolyzed pneumococci intravenously in amounts sufficient to provoke reactions, usually 10 to 20 c.c. The temperature generally dropped 2° to 6° F., followed by a rise to its former level or slightly lower. In a series of 35 cases, chills were noted in 12. Since it was impossible to control the occurrence and degree of the chills and since the beneficial effects were transient and the course of severe cases uninfluenced, this method was abandoned, as the possible beneficial effects were more safely secured by subcutaneous injections.

Non-specific Vaccine Therapy of Pneumococcus Pneumonia.—Miller⁵ has summarized his experience with 15 cases as follows:

"Fifteen consecutive patients with lobar pneumonia entering Cook County Hospital were treated by a single intravenous injection of typhoid vaccine. The dosage used was 30,000,000, the minimum amount required to give a chill. All reacted by a rise in temperature and a leukocytosis. In 9 patients the vaccine did not modify the course of the disease. In 6 the patient was detoxicated following the injections. The pulse, temperature, and respiration returned to normal, the cough and pleural pain subsided, and the patient stated that he felt much better. In 3 of the 6 cases the improvement was temporary, as after the lapse of from twelve to twenty-four hours the symptoms returned with unmodified severity. In 3 cases

¹ Jour. Amer. Med. Assoc., 1915, 64, 2160.

² Jour. Amer. Med. Assoc., 1921, 76, 178.

³ New York Med. Jour., June 8, 1918; Penna. Med. Jour., 1919, 12, 506; Jour. Amer. Med. Assoc., 1919, 73, 1741.

⁴ Jour. Amer. Med. Assoc., 1916, 67, 1929.

⁵ Jour. Amer. Med. Assoc., 1920, 74, 1598.

the detoxication was permanent; however, the patients had a moderate temperature for from three to four days, to the time at which the crisis would normally appear. They were, however, entirely free from evidence of intoxication. There was no relation between the severity of the chill, the temperature reaction and degree of increased leukocytosis, and the beneficial results of the vaccine."

As stated by Petersen these results suggest a temporary detoxication that leaves the general course of the disease process unaltered. Very probably the effects are purely non-specific in which antibodies play no part, inasmuch as the disease has too short a period of incubation to permit the production of antibodies in time to materially influence the course of the disease during the first week.

Cowie¹ has reported favorably upon the results of intravenous injection of typhoid vaccine in the treatment of influenzal pneumonia, providing the injection is given before the fourth day, finding that the acute symptoms may subside in from one to three days. Cases showing undoubted evidence of advanced myocardial insufficiency or acute endocarditis should not receive these injections.

Leukocytotherapy of Pneumococcus Pneumonia.—Hess² has reported the treatment of 7 cases of lobar pneumonia by the daily subcutaneous injection of 10 c.c. of leukocytic extract. In every instance the injection was followed by a drop in temperature and a decrease of the toxic symptoms, although the disease was not materially shortened. Floyd and Lucas³ have reported favorably upon the results of this treatment in 41 cases of lobar pneumonia, and particularly the beneficial effect upon the pulse and respiration. Lambert⁴ has likewise observed good results in a small series of cases. Williams and Youland,⁵ however, were unable to see any beneficial effects in a series of 7 cases receiving amounts of extract varying from 20 to 320 c.c. by subcutaneous injection per day. The temperature, leukocyte count, general condition of the patient, course, and termination of each case did not appear to be influenced.

It would appear that the direct bactericidal activity of leukocytic extracts is feeble and that beneficial effects are to be ascribed to non-specific agencies. For this purpose 10 to 15 c.c. may be administered per day by subcutaneous injection.

TREATMENT OF INFLUENZAL AND STREPTOCOCCUS BRONCHOPNEUMONIA

Etiology of Bronchopneumonia.—The bacteriology of bronchopneumonia is quite complex. The disease is caused principally by a group of organisms that are not as invasive as pneumococci, but gradually creep down the bronchial tree and involve patches of lung by direct involvement of the bronchioles. For this reason bronchopneumonia is usually a complication developing in diseases of the upper respiratory tract as whooping-cough, influenza, measles, grip and the "common cold," and especially acute bronchitis. From the etiologic standpoint the influenza bacillus, streptococci, Type IV pneumococci, Staphylococcus aureus and Micrococcus catarrhalis are of most importance.

Influenzal Pneumonia; Bacterium Pneumosintes.—The pneumonia of influenza may be caused by a variety of micro-organisms and mainly Bacillus influenzæ, streptococci, and staphylococci. The pneumonic lesions

¹ Jour. Amer. Med. Assoc., 1919, 72, 1117.

² Jour. Med. Research, 1908, 14, 325.

³ Jour. Med. Research, 1909, 21, 223.

⁴ Amer. Jour. Med. Sci., 1909, 137, 506.

⁵ Jour. Med. Research, 1914, 31, 391.

have seldom revealed *B. influenzae* in pure culture. It is highly probable that in influenza resistance is greatly lowered to secondary infections with these other micro-organisms.

Direct evidence of this has been apparently furnished by the studies of Olitsky and Gates.¹ These investigators have observed that the intratracheal injection of rabbits with the nasopharyngeal secretions of influenza cases collected within thirty-six hours after the onset of the disease, produces severe injury of the lung tissue with edema and hemorrhages and favoring the production of severe pneumonia by ordinary bacteria. Filtrates of these secretions have produced similar results and anaerobic cultures of the filtrates in the Smith-Noguchi ascites broth kidney medium have developed a minute bacillary body to which has been given the name *Bacterium pneumosintes*—a bacterium that injures the lung.

Rabbits recovering from intratracheal injections of this filter-passing organism or of nasopharyngeal secretions of influenza cases are apparently immune to reinoculation. Injection of rabbits with cultures results in the production of specific antibodies, and recently antibodies for *Bacterium pneumosintes* were found in the blood of 17 of 19 human cases of influenza. These studies indicate that *Bacterium pneumosintes* may be the primary etiologic agent of influenza, but further experience is required before a final statement may be made.

Characteristically this is a broncho- or nodular hemorrhagic pneumonia. As shown by Blake and Cecil² experimentally in monkeys the influenza bacillus is capable of setting up a bronchitis and broncholitis with peribronchial consolidation—a true bronchopneumonia with hemorrhage and edema, sometimes succeeded in the later stages by emphysema and bronchiectasis. In human infections, however, other bacteria are usually present and it is often difficult to determine just what part of the process is due to the influenza bacillus.

According to Blake and Cecil, the influenza bacillus travels down the bronchial tree into the bronchioles and involves the neighboring tissues by direct contiguity, producing patches of inflammation probably by means of exogenous toxins. The bacillus does not appear to enter the lymphatic channels as does the pneumococcus with subsequent wide-spread distribution and more general involvement of the lung tissue.

Streptococcus Pneumonia.—This pneumonia may develop in the course of influenza, measles, and other infections; apparently it may also occur spontaneously and without predisposing causes. According to Cecil³ it is the organism most frequently found in bronchopneumonia and may be classified into the "hemolytic" and "viridans" group.

Streptococcus hemolyticus produces a severe form of lobular pneumonia, frequently complicated by multiple abscesses and empyema with a mortality of about 50 per cent.

Streptococcus viridans produces a mild pneumonia with a mortality of less than 10 per cent.

The experiments of Blake and Cecil have indicated that *Streptococcus hemolyticus* pneumonia is produced in much the same manner as pneumococcus pneumonia, that is, by entering the lymphatics at the root of the lung and gradually involving the lung tissue, as well as by extension along the connective-tissue framework.

¹ Jour. Exper. Med., 1921, 33, 125, 361, 373, 713; *ibid.*, 1921, 34, 1; *ibid.*, 1922, 35, 1, 553, 813; *ibid.*, 1922, 36, 685.

² Jour. Exper. Med., 1920, 32, 401.

³ Amer. Jour. Med. Sci., 1922, 164, 58.

Staphylococcus Aureus Pneumonia.—This pneumonia has been described by Chickering and Park¹ as a complication of influenza during the recent pandemic. Of 1409 cases of pneumonia in Camp Jackson during 1918, 153 were found to be associated with this organism. The striking clinical features of the disease were the "peculiar cherry-red indigo-blue cyanosis, the fulminating course, the lack of definite signs of consolidation of the lungs, the dirty salmon-pink purulent sputum, and usually a leukopenia. Pathologically, if the disease is of long enough duration, it is characterized by innumerable abscesses in the lungs. The prognosis is always grave, though a few patients occasionally survive." Only 2 patients in their series recovered.

Treatment of the Bronchopneumonia of Influenza with Antipneumococcus Serum.—Ordinarily serum has not been employed in the treatment of bronchopneumonia. During the pandemic of influenza, however, the incidence and mortality of pneumonia was so high as to lead to numerous trials of serum treatment.

At Camp MacArthur pneumococci, and especially Type IV, was found so frequently in 440 cases (85.8 per cent.) that polyvalent pneumococcus serum was employed in treatment. Medalia and Schiff² report that of the serum treated cases, omitting those that received serum in a moribund condition, the mortality among 286 cases was 7.6 per cent., while among 277 cases not treated with serum, the mortality was 24 per cent. It was believed that the serum treatment had been of value.

Treatment of the Bronchopneumonia of Influenza with Human Convalescent Serum and Blood.—Toward the close of the pandemic of influenza considerable attention was being given the treatment of pneumonia by injections of serum and citrated blood from convalescent human beings.

McGuire and Redden³ in 1918 reported upon the successful treatment of a group of 37 cases by this method. Donors were selected from among adult convalescent cases about seven to ten days after subsidence of fever and about 800 c.c. of blood removed from each under aseptic conditions. Wassermann and blood grouping tests were then conducted. Treatment was conducted by the intravenous injection of 75 to 125 c.c. of homologous serum followed by reinjections at intervals of eight to sixteen hours until the patient improved; the average amount of serum for each case was 300 c.c.

Sanborn⁴ treated 101 cases of bronchopneumonia in influenza with convalescent human serum. The dosage for adults was 100 c.c. and for children 50 c.c. From one to six doses were given each patient according to indications, at eight- to twenty-four-hour intervals. Those patients only were used as donors who had recovered from an undoubted bronchopneumonia which had followed epidemic influenza. Of the 67 patients who recovered, 59 required one or two doses; 7, three, and 1, six. The maximum dosage of serum in a given case in the recovered group was 700 c.c. Of 34 who died, 17 who received one dose did not live a sufficient length of time to receive a second. The remaining 17 received two to four doses and 2 then received 500 c.c. and 700 c.c. respectively as the total dosage. Reactions following serum were conspicuous by their absence. There were practically no complications in recovered cases. The mortality rate in this series of 101 serum treated cases was 33.6 per cent. In 184 cases of influenzal pneumonia not treated by serum, the mortality was 21 per cent. Sanborn

¹ Jour. Amer. Med. Assoc., 1919, 72, 617.

² Jour. Amer. Med. Assoc., 1918, 71, 1821; Boston Med. and Surg. Jour., March 20, 1919.

³ Jour. Amer. Med. Assoc., 1918, 71, 1311; Amer. Jour. Public Health, 1918, 8, 741.

⁴ Boston Med. and Surg. Jour., 1920, 18, 171.

maintains that the mortality rate for this series was not a measure of the value of convalescent serum, but was an index of the severity of the disease in the group as a whole. Convalescent serum appeared to have value when it was administered during the first three days of the pneumonia. Its value rapidly decreased when administered after the third day of the pneumonia as indicated by rapidly increasing mortality rates, according as administration was delayed from day to day. The time factor, that is, the period between onset and day of treatment, had a close relation to the mortality rate and seemed largely to determine success or failure.

Francis, Hall and Gaines¹ have also expressed the opinion that intramuscular injections of 50 to 100 c.c. of convalescent serum proved helpful in the treatment of the bronchopneumonia of influenza.

Maclachlan and Fetter² have treated 54 cases of bronchopneumonia of influenza with intravenous injections of 75 to 100 c.c. of blood from non-syphilitic convalescent cases; the mortality was 27 per cent. This amount of blood was removed into 40 c.c. of 2 per cent. sodium citrate in saline solution and injected without attention to compatibility of the bloods of donor and recipient. Reactions of chills and fever occurred in some instances, but no other ill effects were observed. Ross and Hund³ have also treated a group of cases with intravenous injections of 250 to 500 c.c. of citrated blood taken from compatible donors among convalescent cases of influenza; the mortality rate was 21.4 per cent. as against 42.8 per cent. among cases symptomatically.

Treatment of the Bronchopneumonia of Influenza with Antistreptococcus Serum.—Rosenow⁴ has employed intravenous injections of 25 to 100 c.c. of monovalent antistreptococcus serum prepared by the immunization of horses with streptococci recovered from cases of influenza during the epidemic. Of 12 patients treated by him, all but one were critically ill at the time of serum treatment. Five recovered and 7 died. Three of the patients who died were practically moribund at the time of the serum treatment and good effects could scarcely be expected. Two others that died showed green-producing streptococci immunologically different from the strain with which the serum was prepared, and in 2 hemolytic streptococci caused death. In these cases also improvement could not be expected. In all cases in which specific agglutination was obtained marked improvement followed the injection of the serum, and in no case were good effects noted at a time when agglutination tests were negative.

Edgewood⁵ and Hughes⁶ have also reported favorable results from the use of antistreptococcus serum.

Treatment of the Bronchopneumonia of Influenza with Non-specific Agents.—Intramuscular injections of 5 to 20 c.c. of sterile *milk* for one to three injections have been employed by Von den Velden,⁷ Münzes and Ptitz,⁸ Patschkowski,⁹ and others; satisfactory results are claimed. Borchardt and Ladwig¹⁰ have employed intravenous injections of small amounts of 5 to 10 per cent. solutions of *sodium chlorid* in the treatment of 98 cases, and remark particularly on the detoxication of severe cases. Lüdke has treated 10 cases with intravenous injections of 1 to 3 c.c. of 10 per cent. solutions of *albumose*. In 5 of these an immediate improvement was noted;

¹ Military Surgeon, 1920, 67, 177.

² Jour. Amer. Med. Assoc., 1918, 71, 2053.

³ Jour. Amer. Med. Assoc., 1919, 72, 640.

⁴ Jour. Infect. Dis., 1920, 26, 614.

⁵ Brit. Med. Jour., 1918, 2, 515.

⁶ Lancet, 1919, 2, 782.

⁷ Deutsch. med. Wchn., 1918, xlv, 1446.

⁸ Münch. med. Wchn., 1919, 66, 227.

⁹ Münch. med. Wchn., 1916, 66, 531.

¹⁰ Berl. klin. Wchn., 1920, 57, 1123.

in 2 cases pneumonia developed with empyema, and in 3 the temperature came down only after a number of injections.

Subcutaneous injections of turpentine (0.1 to 1 c.c. of 20 per cent. solutions in sterile olive oil) for the production of fixation abscesses have been claimed by Tailens,¹ Netter,² Pehu and Pillon,³ Hodel,⁴ Probst,⁵ and others to yield good results probably by the production of leukocytosis in influenza and particularly its bronchopneumonia in children.

TREATMENT OF DIPHTHERIA

SERUM (ANTITOXIN) TREATMENT OF DIPHTHERIA

The discovery of diphtheria antitoxin and its use in the treatment of this infection constitute one of the triumphs of modern medicine.

Twenty-five years ago diphtheria was one of the most dreaded of diseases, accompanied ordinarily by a mortality of at least 30 per cent., while the loss of life from the laryngeal form of the disease, particularly after tracheotomy, was simply appalling.

Shortly after Roux and Yersin (1888) had demonstrated that the symptoms of diphtheria were due largely to a soluble poison or toxin secreted by the bacilli, Ferran, and later Fränkel and Brieger (1890), undertook experiments in active immunization against diphtheria. About the same time von Behring discovered the antitoxin, and in a series of extensive researches with Wernicke he established experimentally its prophylactic and therapeutic value in diphtheria. The first attempt to apply this discovery to the cure of this infection of the human being was made in von Bergmann's clinic (1891). The results, while encouraging, were not altogether satisfactory, owing largely to the fact that the serums were weak and the doses given too small. The discovery, however, resulted in creating an extraordinary stimulus to researches in immunity, and during the following two years more powerful serums were prepared, so that in 1896 a marked drop in the mortality of diphtheria was apparent in those places where the antitoxin was being used.

Since then diphtheria antitoxin has been the means of saving countless thousands of lives, and the treatment of diphtheria, instead of being a reproach to medicine, has become the model of what the scientific treatment of an infectious disease ought to be. Statistics and the individual experiences of those especially engaged in the treatment of diphtheria show that when the antitoxin is used on the first day of the disease, practically no mortality occurs. Parents and guardians should be taught this fact, and cautioned to seek medical advice promptly whenever a child complains of sore throat. While the use of diphtheria antitoxin is still decried and opposed by a few members of the medical profession—and this is not to be wondered at when it is remembered that cowpox vaccination still has its opponents—it is at least to be hoped that no physician will deprive a patient suffering from diphtheria of the benefits to be derived from the antitoxin treatment. *In the absence of special contraindications the refusal or neglect to use antitoxin in the treatment of diphtheria would, in the opinion of most physicians, constitute an act of criminal negligence and malpractice.*

Preparation of Diphtheria Antitoxin.—The methods of preparing and standardizing diphtheria antitoxin are given in Chapter XIII. Since antitoxin gradually deteriorates with time, physicians should carefully observe

¹ Arch. d. méd. d. enf., 1919, 22, 527.

² Bull. Acad. d. Méd., 1918, 80, 427.

³ Lyon méd., 1913, 121, 941.

⁴ Cor.-Bl. f. Schweiz. Aerzte, 1919, xlix, 310.

⁵ Rev. méd. d. l. Suisse rom., 1920, xl, 159.

the date printed upon each package of antitoxin, which is the time limit calculated by the manufacturers beyond which they do not guarantee that the full antitoxic strength is maintained.

Nature of Diphtheria.—In the great majority of cases diphtheria is a local infection of some portion of the upper respiratory tract. The bacilli are usually inhaled, find lodgment upon a mucous membrane, and secrete a toxin that produces necrosis of the cells of the mucosa and effectually resists phagocytosis of the bacilli. From this area of infection, which now becomes a prolific source of toxin production, the toxin or poison is absorbed by the body fluids, and the resulting toxemia is chiefly responsible for most of the symptoms of the disease.

Other micro-organisms, such as staphylococci, pneumococci, and streptococci, which may be unable to infect a healthy mucous membrane, readily multiply in the necrotic tissue and add to the severity of the local lesion, the lymphadenitis, and the toxemia.

Rarely the diphtheria bacilli gain access to the blood-stream. The severity and danger of diphtheria are dependent primarily upon the strength and amount of toxin produced by the bacilli, and secondarily upon the size and location of the primary lesion and the amount of antitoxin present in the patient's blood. A lesion in the larynx is far more dangerous than one of equal size on a tonsil, because in the former the edema and necrotic exudate obstruct the trachea and may produce death by suffocation. On the other hand, the size of the local lesion alone is not an indication of the severity of the infection, because virulent bacilli in a small patch may produce more toxin than less virulent ones in a larger area, and the degree of local tissue necrosis is not an absolute indication of the toxicity of the soluble poison. Other things being equal, the patient who has most antitoxin, either naturally or acquired as the result of a previous injection with antitoxin or of an attack of diphtheria, will present least evidences of toxemia, although the bacilli causing the infection may be most virulent. For example, the highly virulent strain of diphtheria bacillus used extensively in the past twenty years in the production of antitoxin was isolated by Park and Williams from the throat of a patient presenting no clinical symptoms other than redness of the fauces and slight toxemia.

The primary lesion of diphtheria is usually located in the throat (tonsils, uvula, larynx), and frequently in the nose; more rarely the ears, conjunctiva, vulva, prepuce, and wounds are the seats of primary infection.

Purposes of Serum Treatment of Diphtheria.—If we were always certain of seeing our patients on the first day of their illness, and if the disease could always be diagnosed in this stage, the treatment of diphtheria would resolve itself into an immediate dose of antitoxin and rest in bed two or three weeks. But patients frequently come under treatment comparatively late in the disease, or the true nature of the condition may not be fully diagnosed at first and treatment thus be delayed. Diphtheria is, therefore, still to be regarded as a dangerous infection, and while the proper use of antitoxin constitutes the most important part of the treatment, local applications, general constitutional measures, and the management of the various conditions that may complicate the disease are all to be considered. Here we will discuss only the serum treatment of the disease.

Bearing in mind the pathology of diphtheria, serum treatment aims to fulfil the following primary indications:

1. To neutralize all free toxin circulating in the body fluids, and also to neutralize, as much as possible, the toxin that has already united with the tissue cells.

2. To cause the destruction or removal of the bacilli producing the toxin as quickly as possible.

3. To furnish the patient with sufficient excess of antitoxin to neutralize the toxin as rapidly as it is produced until the virulent bacilli disappear.

Action of Diphtheria Antitoxin.—Diphtheria antitoxin best fulfils these requirements. In fact, there are no substitutes. In former days the powerful and irritant caustics and germicides that were freely applied to the throat, instead of limiting the disease and destroying the bacilli, probably actually encouraged its extension by excoriating and depressing the resistance of the surrounding mucous membranes.

1. The chief action of antitoxin is just what its name implies, namely, a substance that neutralizes the toxin. This is regarded as a chemical reaction analogous to the neutralization of an acid by an alkali. When the antitoxin molecule has united with the toxin molecule, it is believed that the toxin is neutralized and that both are rendered inert. As the result of experimental studies, however, we know that this union is not always a firm one, and it is possible for the toxin to become dissociated and attack body cells or other molecules of antitoxin, thus explaining in part the necessity for giving quite large doses of antitoxin—doses that are out of all proportion to what we would expect to be necessary, when considered weight for weight between guinea-pig and man, to effect complete neutralization of the toxin.

It is reasonable to presume, and may be accepted as true, that a stronger affinity exists between diphtheria toxin and antitoxin than between body cells and toxin. Just as the union between this toxin and its antitoxin is somewhat unstable, so, in like manner, it is probable that the union of toxin and body cells is not so firm but that it may, during an early stage, be dissociated to some extent by the more attractive antitoxin. This factor is to be borne in mind in the treatment of diphtheria, and is an additional argument for the administration of large doses of the serum.

2. Antitoxin pure and simple does not, however, constitute the only factor of value in antidiphtheric serum. While the pure antitoxin neutralizes the toxin, it has no injurious action on the bacilli themselves, and, indeed, it is said that the bacilli may live and multiply in the presence of large amounts of antitoxin. How, then, are we to explain the gradual disappearance of the membranous exudate and the bacilli at the local site of infection? It has been shown experimentally that virulent diphtheria bacilli resist phagocytosis. This condition is probably due to an actual leukotoxic action of the diphtheria toxin, aided by an aggressin-like action of the toxin which neutralizes opsonin, and in this manner prevents phagocytic activity. The writer in common with other observers, has shown that the antiserum as ordinarily produced neutralizes the antiphagocytic action of diphtheria bacilli, and enables the polynuclear leukocytes to ingest them readily.¹ Whether this is brought about through neutralization of the toxin by antitoxin, or is due to the presence of an immune opsonin (bacteriotropin) that lowers the resistance of the bacilli, or to the presence of an anti-aggressin that neutralizes the intrinsic defensive mechanism of the bacilli and thus favors phagocytosis, I am unable to state, but probably all three factors are operative.

3. As ordinarily prepared, diphtheria antitoxin possesses little or no bacteriolytic activity. I have found, however, that antitoxin will fix complement with an antigen of diphtheria bacilli,² indicating, therefore, the presence of bacteriolytic amboceptors. Serums produced by immunizing

¹ Jour. Med. Research, 1912, xxvi, 373.

² Jour. Infect. Dis., 1912, xi, 44.

horses with unfiltered cultures of diphtheria bacilli instead of with the filtered toxin alone have been advocated for the general and the local treatment, in the hope of securing lysis of the infecting bacilli. Certainly it would appear wise to raise the bacteriotropic and bacteriolytic content of an immune serum by injecting the horses occasionally with unfiltered cultures, for it is highly probable that the action of the antiserum depends not only upon an antitoxin but also, to some extent at least, upon a bacteriotropin and possibly a bacteriolysin.

Subcutaneous Administration of Diphtheria Antitoxin.—Antitoxin is usually administered by *subcutaneous injection* into the tissues of the back, abdomen, or buttocks. Experimental studies have tended to show that complete absorption does not occur until forty-eight hours after, although it is common clinical experience to observe improvement take place during the first twenty-four hours after injection.

Park¹ has proved that it takes twenty-four hours for the major part of the antitoxin to be absorbed by the blood from the subcutaneous tissues and some twelve hours from the muscles; two or three days are required for total absorption.

As will be emphasized later, *it is highly desirable and necessary to get antitoxin into the circulation as soon as possible after infection has occurred.* Usually this is best accomplished by giving antitoxin to every patient even suspected of being diphtheric, and making the diagnosis afterward; the next best method is to administer the antitoxin in such manner as to favor quick absorption. *For this reason intramuscular and intravenous injection should be resorted to in all severe cases.* As previously pointed out the Schick reaction in diphtheria has indicated that diphtheria toxin may be dissociated from tissue cells by large doses of antitoxin. Park and his associates have shown experimentally by this reaction that 20,000 units of antitoxin given subcutaneously were necessary to yield an effect equal to 1000 units given intravenously.

The subcutaneous route, therefore, should be used only in the treatment of mild infections. Intramuscular injections are just as easy, not more painful, and, as will be discussed in the succeeding paragraph, serum is absorbed at least three times more quickly.

Intramuscular Administration of Diphtheria Antitoxin.—Antitoxin does not become effective until it is absorbed in the blood and then passing through the capillary walls to the tissue fluids and cells. The greater the quantity in the blood, the more thorough and rapid will be the neutralization of toxin.

As previously stated, *intramuscular injections are easily given in the muscles of the thighs and buttocks, and since absorption is three to four times more rapid than by subcutaneous injection, there is no valid reason why intramuscular injections should not be used routinely instead of subcutaneous injections; particularly in the treatment of late cases and cases of more severe infections.* Rolleston and MacLeod,² Veeder,³ and numerous others have emphasized the value of intramuscular injections.

Intravenous Administration of Antitoxin.—*Intravenous injections* are far more difficult, especially in children with fat arms and feeble circulations. An anesthetic or ten minutes' struggling may do the patient harm, and unless the injection can be given with little disturbance and danger, the serum should be given by intramuscular injection. Not infrequently,

¹ Jour. Amer. Med. Assoc., 1912, 58, 1976; Jour. Infect. Dis., 1914, 14, 338.

² Brit. Jour. Dis. Child., 1913, 11, 289.

³ Missouri State Med. Assoc. Jour., 1915, 12, 145.

however, an intravenous injection yields splendid results in severe and apparently hopeless cases, and in older children and adults this route of administration should be considered.

There can be no doubt, however, of the value of intravenous injections in severe cases and especially in late advanced cases. By these are meant cases already sick for twenty-four to seventy-two hours with rapid extension of the disease in the throat or nose, swelling of the tissues and glands, and severe constitutional symptoms. According to Park¹ antitoxin given intravenously passes out to the tissues about ten times as rapidly as when given subcutaneously and four times as rapidly as when given intramuscularly.

Intravenous injections are being given in increasing frequency; numerous reports advocating this route in the treatment of severe infections, whenever possible, have been made by Tachan,² Nickolson,³ Dupaquier,⁴ Schone,⁵ Mixsell,⁶ Schorer,⁷ Alber,⁸ and others. If it were not for the fact that the technic of administration is more difficult and the reactions sharper and more dangerous, the intravenous route would be the one of choice in the treatment of all but mild early cases.

In laryngeal diphtheria, however, the intravenous injection of antitoxin while giving a quicker response, does not greatly lower the death-rate. In a series of 79 cases treated by Park and Mixsell with intravenous injections the mortality was 53.1 per cent. Bronchopneumonia developed in 45.5 per cent. of the former series and in 51.9 per cent. of the latter, and out of a total of 77 cases developing this dreaded complication only 5 survived.

Intravenous injections are apt to be followed in from fifteen to sixty minutes by a rise in temperature of 2° to 5° F. and a chill. The chill is not usually severe, lasts for five to forty-five minutes, and occurs in about 40 per cent. of cases. The temperature falls in a few hours to its former level or below and no bad effects are noticeable. These reactions are doubtless caused by the serum proteins producing a "protein shock reaction," they are not dangerous, but are disturbing at the time and may impress the family unfavorably.

The syringe method is well adapted for the intravenous injection of antitoxin, as the bulk method of serum is usually small, especially if a concentrated antitoxin is being used.

The *technic* of these injections has previously been described in Chapter XXXVII.

Oral Administration.—Antitoxin has also been given by the *mouth* and even by *rectal injection*. The presence of a preservative, usually phenol, renders the oral administration objectionable.

McClintoch and King⁹ have shown experimentally that antitoxin is absorbed after oral administration, but in an irregular and unreliable manner. If digestion is inhibited absorption is facilitated, and they have recommended that 1 minim of fluidextract of opium and 4 to 10 minims of saturated solution of salol in chloroform be added to each dose and that no food be taken for at least four hours before administering the mixture.

¹ Trans. Cong. Amer. Phys. and Surg., 1916, 10, 118.

² Therapie der Gegenwart, 1910, 51.

³ Bull. Med. and Chir. Faculty of Maryland, 1914, 8, 45.

⁴ New Orleans Med. and Surg. Jour., 1915, 68, 145.

⁵ Deutsch. Arch. f. klin. Med., 1913, 110.

⁶ Californ. State Jour. Med., 1913, 11, 297.

⁷ Amer. Jour. Dis. Child., 1915, 9, 59.

⁸ Jahrb. f. Kinderh., 1913, 30, No. 2.

⁹ Jour. Infect. Dis., 1906, 3, 701; *ibid.*, 1909, 6, 46.

While a therapeutic effect may be secured after large doses have been swallowed, there are very few occasions when this should be the method of choice.

Serum sickness does not usually follow oral administration and the method may be of value in the treatment of diphtheria occurring in an individual extremely susceptible to horse-serum. Furthermore, the method may be of value for continuing the effects of antitoxin when by reason of fear on the part of the patient or for other reasons injections cannot be given.

Local Application of Antitoxin.—There does not appear to be any advantage in applying either fluid or dried serum antitoxin to the involved tissues in the nose or throat. The antitoxin itself is not directly bactericidal and the only good to be expected is that of cleansing, which may be done just as well with simple saline solution.

Importance of Early Treatment.—*The most important point to be observed in the treatment of diphtheria is to give antitoxin at once. It may be fatal to wait for the result of a culture, except perhaps in the case of the mildest of infections. In fact, the necessity for early administration of antitoxin cannot be overestimated. When once suspicion is aroused, antitoxin should be given at once and the diagnosis may follow. A few hours may make an enormous difference in the prognosis of any case, and while a dose of 2000 units of antitoxin may prove of the utmost value in checking diphtheria, it will do no harm whatever in case the disease is not present.*

It is true that a physician will naturally hesitate to administer antitoxin unnecessarily; nevertheless, diphtheria is frequently a difficult disease to diagnose clinically, and is quite likely to be mistaken for tonsillitis. For this reason many physicians prefer to wait for the result of a culture, and this is proper, provided that the patient, especially in the case of a child, is given the benefit of the doubt by receiving 2000 units of antitoxin. *It is to be emphasized that a single negative culture does not exclude diphtheria. As ordinarily made, about 20 per cent. of primary cultures from genuine cases of diphtheria fail to show the presence of bacilli, whereas subsequent cultures will show them to be present in large numbers. A primary negative culture is most likely to be obtained from a patient having a heavy exudate, as the physician may rub lightly over the membrane, culturing the micro-organisms of secondary infection and overlooking the diphtheria bacilli in the depths of the membrane adjacent to the diseased mucous membrane. To wait another twenty-four hours for a second culture still further reduces the patient's chances for recovery. In the vast majority of instances, therefore, antitoxin should be given at once and repeated as often as is necessary until the correct diagnosis is established, and not one but at least two successive negative cultures should be obtained before diphtheria is to be excluded with any reasonable degree of safety.*

The following table, compiled from the annual reports of the Philadelphia Hospital for Contagious Diseases, shows the decided influence of early treatment upon the mortality of diphtheria. This table comprises cases of diphtheria alone, and does not include cases complicated by other diseases, such as scarlet fever and measles. *It is worthy of special notice that of 741 cases treated with antitoxin on the first day of the disease, not one died.* Ker,¹ in an exceptionally rich experience, has never seen a fatal result occur in a case that developed in a hospital and in which antitoxin was administered on the first day of the disease.

Recently he has published the following table, in which the effects of early treatment upon the mortality is brought out very clearly:

¹ Infectious Diseases, 1920, Oxford Med. Press.

MORTALITY OF DIPHTHERIA ACCORDING TO DAY ON WHICH ANTITOXIN WAS FIRST INJECTED IN 8591 CONSECUTIVE CASES

DAY OF ILLNESS.	NUMBER OF PATIENTS.	DEATHS.	PERCENTAGE OF MORTALITY.
First.....	329	5	1.52
Second.....	2269	77	3.39
Third.....	2407	165	6.85
Fourth.....	1612	176	10.91
Fifth.....	911	136	14.92
Sixth.....	416	54	12.98
Seventh.....	320	53	16.56
	327	50	15.29
Totals.....	8591	716	8.33

MORTALITY OF DIPHTHERIA ACCORDING TO THE DAY OF ADMISSION (WHICH ALSO INCLUDES THE TIME OF GIVING THE ANTITOXIN) IN THE PHILADELPHIA HOSPITAL FOR CONTAGIOUS DISEASES

YEAR.	TOTAL NUMBER OF CASES.	MORTALITY ACCORDING TO THE DAY ON WHICH ANTITOXIN WAS FIRST INJECTED.							AVERAGE MORTALITY IN DIPHTHERIA ALONE.
		First Day.	Second Day.	Third Day.	Fourth Day.	Fifth Day.	Sixth Day.	After Sixth Day.	
1904.....	712	4.09	13.72	17.54	14.75	19.44	12.94	10.81
1905.....	862	4.43	9.22	16.66	13.04	9.52	22.89	10.09
1906-07.....	1,239	3.45	12.90	10.85	13.08	29.41	6.09	8.70
1908.....	1,426	6.62	4.7	10.60	11.71	21.43	23.96	8.55
1909.....	2,153	4.61	7.13	10.72	9.35	7.25	13.33	6.60
1910.....	1,870	5.50	5.13	8.41	13.74	6.04	8.33	6.42
1911.....	1,895	6.91	9.41	7.12	11.04	7.22	2.66	6.86
1912.....	1,676	2.92	6.33	8.0	9.53	14.97	9.63	6.02
1913.....	1,273	3.92	6.51	6.52	6.87	4.76	12.5	18.8	7.63
Average.....	13,106	0.4	5.0	8.3	10.7	11.2	14.2	13.1	7.96

It is generally believed that the paralyzes of diphtheria are due to a toxone, and not to the true toxin. Ehrlich believes toxone to represent a late secretory product of the diphtheria bacillus, whereas others regard it as a modified toxin. It is certainly apparent, however, that the bacilli should be gotten rid of as soon as possible, so as to eliminate the possibility of toxone production. This is best accomplished by the early use of large doses of antitoxin, aided possibly by judicious local treatment.

Dosage of Diphtheria Antitoxin.—While it is now generally agreed that the doses of from 100 to 200 units, such as were commonly given during the early years of antitoxin therapy, were far too small, there is still some difference of opinion regarding the proper doses to employ. Since the severity of the disease varies so markedly, no hard-and-fast rules can be given. The physician who has a clear idea of the nature of diphtheria and of the action of antitoxin, and knows what to expect of the latter in the treatment of the disease, should have no difficulty in properly treating a case of diphtheria.

General Treatment.—It is to be emphasized, however, that while antitoxin constitutes the most important part of the treatment of diphtheria, it is not usually the whole treatment. Absolute rest in bed, a generous diet, combined with the use of tonics and local applications, are all part of the treatment. Special treatment of the laryngeal form of the disease and the treatment of complications are matters of considerable importance that influence the prognosis in a given case. I may mention, in passing, the

value of continuous enteroclysis of normal salt solution during the early periods of severe infections; this appears to dilute the toxins and aid in their destruction and excretion.

In administering antitoxin the physician must be guided by the clinical condition of the patient, as we have as yet no practical laboratory method for estimating the degree of the toxinemia. Treatment may be regarded as satisfactory when—

1. The local patch of exudate has ceased to spread and shows indications of disappearing.

2. The general condition of the patient is improved, *i. e.*, the toxinemia is decreased, the pulse grows stronger and more regular, and the patient feels more comfortable.

The temperature is not a reliable guide, for not infrequently in severe infections it may be normal or subnormal throughout.

So long as the exudate shows no signs of loosening and disappearing, but tends to spread, and so long as the general condition remains unimproved or grows worse, large amounts of antitoxin should be given. No case should be regarded as hopeless until death supervenes.

Every case of diphtheria is to be treated individually, rather than by any set rule. The amount of antitoxin given in the *initial dose*, and in subsequent doses as well, is dependent on the following factors:

1. The situation and extent of the lesion.
2. The general condition of the patient.
3. The day of the disease.
4. The age of the patient.

1. *The Situation and Extent of the Lesion.*—In ordinary *tonsillar diphtheria* in which there is a small patch on one tonsil and which is first seen on the second day of the disease, the initial dose should be at least 5000 units. When the exudate involves the pillars of the fauces, the uvula, the posterior pharyngeal wall, or is well forward on the palate, this dose should be doubled, and 10,000 units be given.

Cases that present laryngeal symptoms in addition to faucial lesions should never receive less than 12,000 units. In well-marked laryngeal diphtheria with dyspnea and partial suffocation at least 20,000 units should be given, preferably by intramuscular or intravenous injection.

Bronchopneumonia is the most dreaded complication of laryngeal diphtheria and is responsible for the majority of deaths. Out of a series of 77 cases studied by Park and Mixsell only 5 recovered, making a death-rate of 93.5 per cent. Of 81 cases of laryngeal diphtheria not developing bronchopneumonia the mortality was 7.4 per cent.

Hogan¹ has recently stated that 82.11 per cent. of the deaths from diphtheria in Baltimore have been due to laryngeal infections, and he makes a special plea for earlier diagnosis, earlier administration of antitoxin, and intubation.

In *nasal diphtheria* a distinction must be made between those cases that exhibit merely a dirty, chronic discharge containing bacilli, in which 2000 units may suffice, and those that present an actual membrane accompanied by well-marked toxic symptoms, when a large amount of serum—at least 10,000 units—should be given. Owing to the ready absorption of toxin by the nasal mucosa a small patch in the nose may be accompanied by severe general toxinemia and frequently requires energetic treatment.

In *diphtheria of the eye, vulva, or of wounds* relatively large doses should be given—at least from 10,000 to 20,000 units.

¹ Jour. Amer. Med. Assoc., 1921, 77, 662.

All these doses must be regarded merely as suggestions for the initial dose in cases seen on about the second day of the disease. Physicians with extensive hospital experience, for example, Woody and McCullom, generally favor large doses, and while in private practice the question of expense and economy may be a factor, the physician will be wise to err on the side of safety and give a little too much serum rather than too little, especially in the first dose, when so much depends upon how soon antitoxin is introduced into the body fluids.

2. *The general condition of the patient*, or the effect which the toxinemia will have on the patient, is highly important in estimating the dosage of antitoxin. A patient who is pale, drowsy, prostrated, and has a weak and irregular pulse; who has large masses of glands around the neck, or who has marked albuminuria, will require a much larger dose than one who presents none of these signs. Two persons of about the same age and suffering from the same lesions may show very different degrees of toxinemia. In the severe cases we must administer the maximum dose and repeat it at suitable intervals until an effect is produced.

3. *The day of illness* on which the patient comes under observation is important in deciding the initial dose of antitoxin. For corresponding tonsillar lesions a dose of 2000 units on the first day may do more good than 5000 units given on the fourth day. Ker gives second-day cases of purely tonsillar diphtheria 3000 units, and adds an additional 1000 on the following day.

4. *If the age of the patient* exerts any influence at all on dosage, it indicates that more antitoxin should be given to children than to adults with corresponding lesions, as the disease is more fatal in children. So far as infants are concerned, Ker seldom gives more than 4000 units at a single dose, which should be an adequate amount when we consider the small size of the patient. Children over one year of age may be given from 5000 to 10,000 or more units, depending upon the location of the lesion and the degree of toxinemia.

Repeating the Dose.—Whether or not subsequent doses of antitoxin will be required is dependent upon the circumstances of the individual case. In ordinary cases if on the day after treatment is commenced there is no diminution in the amount of membrane visible and the general symptoms have not improved, the dose should be repeated. If the membrane has spread and the toxinemia is worse, the second dose should be larger than the first. In septic cases the second dose may be given in from six to ten hours after the first. If the symptoms are less urgent, the interval may be extended to twelve, but should never exceed twenty-four hours.

One thing is certain: a part of what should be the first dose should not be held back to be given later. When the first dose has been of sufficient size, the second and third injections are not required. As previously stated, subsequent injections may result in the neutralization of toxin already united with body cells as indicated by the investigations of Kleinschmidt,¹ but most depends upon a large single dose as early in the disease as possible.

The Importance of Large Doses of Antitoxin.—As to the *total amount of serum to be administered*, continued injections at relatively short intervals are required until improvement has taken place. As long as a membrane is present and the patient is toxic it is probably worth while to push the treatment unless these show a tendency to clear away. Time must be allowed for absorption to take place, and the serum should not be pushed so far as to be wasted, and, quite possibly, excreted unchanged. The remedy is expensive, especially in private practice, and it is obviously desirable to have

¹ Jahrb. f. Kinderh., 1912, 76, 1.

due regard for economy. While, as previously mentioned, physicians of such wide experience as McCullom, of Boston, and Woody, of Philadelphia, frequently give 200,000 or more units in severe cases of diphtheria, others, *e. g.*, Ker, of Edinburgh, have never given more than 64,000 units to a single patient, and, indeed, several of my colleagues of wide experience claim that they have secured excellent results in severe infections with doses that seldom exceeded 10,000 units.

The following table by Park summarizes well these questions of dosage and route of administration:

DOSAGE OF UNITS OF ANTITOXIN IN DIPHTHERIA—SINGLE DOSE ONLY

For infants, 10 to 30 pounds (under two years):			
MILD CASES.	MODERATE CASES.	SEVERE CASES.	MALIGNANT CASES.
(Subcutaneous or intramuscular injection) 2000 to 3000.	(Intramuscular or subcutaneous injection) 3000 to 5000.	(Intramuscular or one-half intravenous and one-half intramuscular or subcutaneous) 5000 to 10,000.	(One-half intravenous and one-half intramuscular) 10,000.
For children, 30 to 90 pounds (under fifteen years):			
3000 to 4000.	4000 to 10,000.	10,000 to 15,000.	10,000 to 20,000.
For adults, 90 pounds and over:			
3000 to 5000.	5000 to 15,000.	10,000 to 20,000.	15,000 to 40,000.

Antitoxin Treatment of Carriers.—Diphtheria antitoxin has no effect upon ridding the tissues of the nose and throat of carrier diphtheria bacilli. Fluid and dried sera have been applied, but have proved ineffectual, although under any plan of treatment a few apparent successes may be encountered. Recent experiments by Gelien, Moss and Guthrie¹ have shown that the subcutaneous injection of antitoxin may not prevent the successful lodgment and growth of diphtheria bacilli in the nasopharynx of individuals so treated.

Treatment of Relapses.—Occasionally after a patient has recovered from an attack of diphtheria the infection recurs after several weeks. It is in such cases that the physician hesitates to administer antitoxin on account of the discomforts occasioned by serum sickness. It is true that serum sickness is more likely to follow in these than in primary cases, and the very profuse and itchy eruption, joint pains, and fever do indeed render the patient quite uncomfortable. Since a relapse is usually, although not always, comparatively mild, the serum may be dispensed with if there is no involvement of the larynx and if there is not much evidence of toxemia; otherwise full doses of antitoxin should be given without hesitation. The subcutaneous injection of 0.5 c.c. of the serum two or three hours before the main dose is given may possibly produce a condition of anti-anaphylaxis and ward off the more dangerous symptoms. If respiratory difficulties should follow a reinjection of serum, adrenalin chorid, atropin, and caffein should be administered hypodermically (see Chapter XXXII).

Antitoxin Sequelæ.—A certain percentage of cases will present a group of symptoms that constitute the condition known as *serum sickness*, occurring at varying times following the administration of antitoxin. This condition has been shown to be due to certain constituents of horse-serum other than the antitoxic antibodies. It is noteworthy that the serum of one horse may cause more serum sickness than that of another; in general, concentrated antitoxins produce fewer cases than whole serum.

¹ Bull. Johns Hopkins Hosp., 1920, 31, 381.

This condition is characterized by the development of a rash (urticarial, multiform, or scarlatiniform), mild fever, joint pains, and possibly adenitis. The scarlatiniform rash may be extremely difficult to differentiate from that of true scarlatina, especially in the wards of a diphtheria hospital, where outbreaks of scarlet fever are not uncommon.

This subject has been considered in greater detail in Chapter XXX. It may be stated here that while the patient is decidedly uncomfortable, and even quite sick, for several days, serum sickness is not a dangerous condition; the treatment is largely symptomatic and palliative.

Value of Diphtheria Antitoxin.—At the present day it seems hardly necessary to introduce elaborate statistics to prove the value of antitoxin in the prophylaxis and treatment of diphtheria.

1. It is generally admitted that most of the reduction in the mortality of diphtheria cannot be attributed solely to the use of antitoxin, for unquestionably bacteriologic diagnosis has permitted the inclusion, in our statistics, of a certain number of cases that, twenty years ago, would not have been classed as diphtheria. Generally speaking, however, the mortality of diphtheria, considering all types of infection coming under observation at varying intervals after the disease has developed, *is at least five times less under antitoxin treatment than when it is treated without antitoxin.* This proportion is true, whether we compare the general mortality before 1896 with the present rate, or whether we take a large city and compare the mortality under both forms of treatment for a single or for several years. For example, in Philadelphia the mortality rates per 100,000 of population for the five years preceding the use of antitoxin were as follows:

1891.....	127.4
1892.....	156.3
1893.....	103.9
1894.....	122.5
1895.....	115.9

In five years following the general use of antitoxin the mortality rates per 100,000 population were as follows:

1906.....	37.78
1907.....	34.60
1908.....	33.35
1909.....	33.6
1910.....	31.7

In Philadelphia, during the years 1909–10 and 1911, the average mortality of diphtheria treated with antitoxin in the Philadelphia Hospital for Contagious Diseases was 9.9 per cent., and in the private practice of physicians, 13.07 per cent. In contrast to these figures is the mortality of 40.34 per cent. in the private practice of those physicians (fortunately few) who refused to give antitoxin or in those families opposed to its use.

Kossel¹ has recently stated that in Germany prior to the use of antitoxin (1886 to 1894) the mortality from diphtheria was from 85 to 130 per 100,000 inhabitants; since 1899 it has never reached 30.

The average mortality in 13,106 cases of pure diphtheria treated with antitoxin in the Philadelphia Hospital for Contagious Diseases during the years 1903–1914 was only 7.96 per cent. As stated elsewhere, when this is compared with the average mortality of about 41 per cent. when no antitoxin was used, it is not difficult to appreciate the therapeutic value of the remedy.

¹ Deutsch. med. Wchn., 1915, 41, 1445.

2. As was previously stated, it is also worthy of note that there is practically no mortality in diphtheria cases receiving antitoxin on the first day of illness. *During nine consecutive years (1904-1913), covering the treatment of 741 such cases in the Philadelphia Hospital for Contagious Diseases, not a single death occurred.* During 1913, 2 of the 51 first-day cases died.

It will also be noted that the patient's chance for recovery grows steadily less with each day that the administration of serum is delayed, and this should be evidence enough to convince any right-minded person that we possess in antitoxin a remarkable remedy for the treatment of diphtheria.

3. The influence of antitoxin is also noted in the mortality of laryngeal diphtheria. While the mortality in this condition is still high, owing to the frequency and dangers of bronchopneumonia and the necessity for operative measures, it has been reduced at least one-half since antitoxin came into general use. Prior to 1896 the mortality was at least 70 per cent.; since then it has been reduced to 35 per cent. or less. As shown in the following table, of 1207 cases treated in the Philadelphia Hospital for Contagious Diseases, the average mortality was 35.6 per cent.

MORTALITY OF LARYNGEAL DIPHTHERIA (INTUBATION CASES) IN THE PHILADELPHIA HOSPITAL FOR CONTAGIOUS DISEASES

(This table excludes those patients dying in the ambulance and moribund cases dying within twenty-four hours.)

YEAR.	TOTAL NUMBER OF CASES.	MORTALITY ACCORDING TO THE DAY UPON WHICH INTUBATION WAS PERFORMED.							AVERAGE MOR- TALITY.
		First Day.	Second Day.	Third Day.	Fourth Day.	Fifth Day.	Sixth Day.	After Sixth Day.	
1903.....	67	66.6	25.3	21.8	30.9	22.5	45.0	26.60
1904.....	125	39.20	29.03	73.68	33.33	70.0	36.84	39.20
1905.....	136	30.76	39.39	50.0	21.42	16.66	42.30	36.76
1906-07.....	72	50.0	55.0	40.74	45.45	71.92	28.57	49.29
1908.....	162	29.26	50.0	33.33	30.76	9.09	38.88	33.95
1909.....	183	50.0	44.18	38.88	23.81	53.84	40.0	42.62
1910.....	89	100.0	55.0	33.33	28.58	50.0	36.46	39.34
1911.....	104	50.0	76.66	55.55	52.63	36.36	27.27	54.80
1912.....	133	30.0	58.33	41.93	48.27	50.0	63.67	50.0	48.87
1913.....	136	100.0	65.91	63.64	35.29	25.0	47.82	68.0	58.82
Average.....	1207	35.6

Formerly when a child contracted diphtheria the parents were warned of the likelihood and danger of the infection involving the larynx and trachea; nowadays this possibility is quite remote.

4. Finally the claim of the opponents of the serum therapy of diphtheria that antitoxin increases the percentage of paralyses is without foundation. While it is true that this percentage is somewhat higher than was noted in former years, this increase is to be explained by the fact that antitoxin saves a larger number of severe cases long enough for them to manifest paralyses, and, second, by the greater attention that has recently been directed to its milder forms. Since diphtheric paralysis is regarded as caused by toxone or a later secondary toxic product of the bacilli, the indications are to rid the patient of the bacilli as quickly as possible, and this is best and most surely accomplished by the proper administration of antitoxin.

Normal Horse-serum and Other Non-specific Agents in the Treatment of Diphtheria.—Bingel¹ has treated 466 cases of diphtheria with subcutaneous injections of normal horse-serum and the results based upon mortality, duration of disease, and number of complications showed no marked differences from 471 cases treated with immune horse-serum (antitoxin). Others have employed normal horse-serum with similar results, the subject having been recently reviewed by Klotz,² and doubtless a part of the beneficial results of the antitoxin treatment of diphtheria are to be ascribed to non-specific effects produced by the serum proteins. This may be one reason why not a few of the older physicians believe that the old-fashioned raw serum antitoxin gave better results than the solutions of concentrated globulins now in common use, although the incidence of serum disease was higher. Calhoun,³ Kraus and Sordelli⁴ found that normal horse-serum had little or no effect upon neutralizing diphtheria toxin injected into guinea-pigs, but this does not exclude the possible beneficial non-specific effects in human beings from injections of normal or antitoxic horse-serum.

Lüdke has treated 15 cases with two to three subcutaneous injections of 3 to 5 c.c. of 10 per cent. solution of albumoses and claims that the results compared favorably with those observed with antitoxin therapy.

Treatment of Diphtheria Carriers with Vaccines and Non-specific Agents.—A large number of substances have been advocated for the treatment of diphtheria carriers to rid the tissues of the upper respiratory tract of both virulent and non-virulent bacilli. Hewlett and Nankivell⁵ have advocated the administration of a vaccine of diphtheria bacilli, and recently Fraser and Duncan⁶ have renewed interest in this subject, advocating the subcutaneous injection of a detoxicated diphtheria bacillus vaccine. The vaccine is so prepared that each cubic centimeter contains 100,000,000,000 and injections are given every four days for twelve injections beginning with 0.05 c.c. in the first dose and gradually increasing the amounts until as much as 3.5 c.c. are given with the twelfth or last injection.

Petersen states that both Paschen and Müller have reported the effective treatment of these cases by intramuscular injections of sterile milk.

TREATMENT OF TETANUS

Tetanus antitoxin was discovered by Behring and Kitasato in 1892. Since then it has proved of great value in the prevention of lock-jaw. While, in general, authoritative opinions regarding its curative value vary, the statistics and the individual experience of many investigators of more recent years show quite conclusively that tetanus antitoxin does possess curative value, and is of distinct aid in the treatment of tetanus, especially when the nature of the disease is understood and the serum is administered accordingly.

Preparation and Standardization of Tetanus Antitoxin.—This technic has been described in Chapter XIII. Since antitoxin gradually deteriorates with time, physicians should carefully observe the date printed on each package of antitoxin, which is the time limit calculated by the manufacturers beyond which they do not guarantee that the full antitoxic strength is maintained.

Action of Tetanus Toxin.—It may be well to recall briefly the main features concerning the pathogenesis of tetanus, as successful treatment depends upon a thorough understanding of these principles.

¹ Deutsch. Arch. f. klin. Med., 1918, cxxv, 284.

² Berl. klin. Wchn., 1919, lvi, 987.

³ Amer. Jour. Dis. Child., 1921, 21, 107.

⁴ Ztschr. f. Immunitätsf., 1921, 31, 107.

⁵ Lancet, 1912, 1, 143.

⁶ Lancet, 1920, 2, 994.

1. Tetanus is a local infection accompanied and characterized by a general toxinemia. The bacilli and spores never gain access to the blood, and are never distributed through the tissues and internal organs, but reside at the local site of infection, where they produce a powerful toxin, which, when absorbed, is responsible for the main lesions and symptoms of the infection. Therefore while the blood of the tetanus patient is sterile, it usually contains the toxin. Neisser has produced tetanus in mice by giving them subcutaneous injections of the blood of a tetanus patient.

2. Tetanus toxin has a strong affinity for nerve tissue, and this constitutes the most important feature in the pathogenesis of the disease. The toxin is rapidly absorbed from the local site of infection into the blood and lymph-streams, where it is distributed to other muscles and reaches the central nervous system indirectly through absorption by the end-plates of motor nerves. As expected, absorption is most likely to occur along the motor nerves supplying the parts injured, and for this reason the muscles and nerves should be infiltrated with antitoxin as soon as possible after an injury has been received.

According to Meyer and Ranson,¹ Marie and Morax,² absorption occurs along the axis-cylinders of motor nerves, the intramuscular endings of which the toxin penetrates. The experiments of Field, Cernovodeanu, Henni and Teale³ indicate, however, that *the toxins are absorbed by way of the lymphatics of the nerves*, and not by way of the axis-cylinders; the latter view is now most general accepted.

Robertson⁴ believes that the toxin passes throughout the body exclusively by the blood and lymph streams and, theoretically, may be neutralized by antitoxin and at any stage in this passage before the final and comparatively indissoluble union with the ganglion cells occurs.

Even though the toxin gains entrance to the blood, it cannot injure the motor nerve tissue directly, as, for example, by means of the blood-vessels supplying the central nervous system. As was previously stated, the toxin in the blood and lymph channels may reach the central nervous system only in an indirect manner, through the end-plates or lymphatics of other motor nerves.

Ascending centripetally along the motor plates and lymphatics, the poison reaches the motor spinal ganglia on the side inoculated; it then affects the ganglia on the opposite side, making them hypersensitive. The visible result of this hypersensitiveness is the highly increased muscle tonus—*i. e.*, rigidity. If the supply continues, the toxin next affects the nearest sensory apparatus: there is an increase in the reflexes, but only when the affected portion is irritated. In the further course of the poisoning the toxin as it ascends continues to affect more and more motor centers and also the neighboring sensory apparatus. This leads to spasm of all the striated muscles and general reflex tetanus (Park).

3. Regardless of the severity of the infection, *there is always an incubation period in tetanus* during which the bacilli multiply and produce toxin which is traveling toward the tissues of the central nervous system. *Antitoxin in sufficient amount will neutralize the toxin as quickly as it is produced, and thus protect the infected individual until the leukocytes and other body cells have destroyed the bacilli and spores.*

In general terms, the severer the wound and the heavier the infection,

¹ Arch. f. Path. u. Phar., 1903, xlix, 369.

² Ann. de l'Inst. Pasteur, 1902, 16, 818; *ibid.*, 1903, 17, 335.

³ Jour. Path. and Bacteriol., 1919, 23, 50.

⁴ Amer. Jour. Med. Sci., 1916, 152, 31.

the shorter will be the incubation period and the higher the mortality. In *acute tetanus* the incubation period is less than ten days; in *chronic tetanus* this period is much longer and more variable.

The toxin is produced, and may be absorbed during or at least soon after the first twenty-four hours following infection; this explains the necessity for administering antitoxin as soon as possible after the injury has been received.

Action of Tetanus Antitoxin.—1. Tetanus antitoxin will neutralize free toxin in a chemical manner similar in some respects to that by which neutralization of an acid by an alkali is effected. It is generally believed that as soon as the molecule of antitoxin has become united with a molecule of toxin, the latter is rendered inert. It may be possible, however, for the toxin molecule to become dissociated and attack nerve-cells or other molecules of antitoxin; this is one reason for the necessity of giving large doses of antitoxin in order than an excess may be at hand.

2. When tetanus toxin has once united with nerve-cells, it is difficult or impossible for the antitoxin to effect its neutralization. Hence the greatest value of the antitoxin lies in prophylaxis; when properly administered, however, it is possible for the antitoxin to aid in the cure of tetanus, and its use should never be omitted in the treatment of any case.

The value of antitoxin in the treatment of tetanus is probably dependent upon the following two factors: (1) Neutralization of all free toxin as quickly as it is secreted and before it is absorbed by the nervous tissue; (2) actual dissociation or neutralization of some of the toxin "loosely united" with nerve-cells or suspended in the lymph after it has left the capillaries and before it is taken up by the nerve-cells.

Of interest in this connection is the question whether different serologic strains of tetanus bacilli exist. This is particularly important because antitoxin is commonly prepared by the immunization of horses with the toxin of a single strain. Since antitoxin has proved so efficacious in the prophylaxis of tetanus during the recent war, it would appear that the antitoxin engendered by one strain will neutralize the toxins of all tetanus bacilli. The question, however, is not definitely settled and the investigations of Tulloch¹ suggest that there may be different strains.

3. Aside from its chief antitoxic action, antitetanus serum probably contains anti-aggressins or bacteriotropins that aid phagocytosis by overcoming their repelling or negatively chemotactic influence. This may, however, be accomplished by simple neutralization of the toxins, which impairs their leukotoxic action sufficiently to permit living leukocytes to engulf and destroy the bacilli.

Methods of Administering Tetanus Antitoxin.—Recent investigations and case reports show quite conclusively that in the treatment of tetanus as much depends upon the method of administering antitoxin as upon the quantity administered.

1. *Absorption by the subcutaneous route is so slow that it should not be depended upon in the treatment of tetanus.* While it is true that the mortality of tetanus has been reduced about 20 per cent. by the administration of large amounts of serum by this route, it should be emphasized that a smaller amount, given subdurally or intravenously, will yield even better results. Knorr has shown experimentally that after subcutaneous injection the maximum quantity of antitoxin is not found in the blood until twenty-four hours have elapsed. Since every hour counts heavily in the chances for recovery when symptoms of tetanus have appeared, it may be laid down as a general rule that the first doses of antitoxin should be given subdurally

¹ Quoted by Bruce, *Lancet*, 1918, 1, 577.

or intravenously. *The subcutaneous route may be chosen when serum is given for prophylactic purposes at the time of injury, but should not be relied upon in the treatment of tetanus.*

2. *Intramuscular injections* may be given to keep up the good effect of antitoxin after the first doses have been given subdurally and intravenously, and are to be preferred to the subcutaneous route whenever the physician is unable to inject the serum subdurally and intravenously.

3. For the rapid neutralization of toxin free in the body fluids serum should be given *intravenously*. In the treatment of tetanus from 10,000 to 20,000 units may be given by this route as early as possible. While it is conceded that when the toxin has become firmly bound to the tissues of the central nervous system dissociation by the use of antitoxin is not possible, yet one can never know, in a given case, how firm this union has become, and clinical results, supported by animal experimentation, show that life may be preserved by large doses of antitoxin injected into the blood.

4. The experimental work of Permin,¹ Park and Nicoll,² supported by the clinical results reported by various observers, shows quite conclusively that the *subdural route is a very efficacious and valuable avenue by which to administer antitoxin in the treatment of tetanus.* The serum should be given by the gravity method, in exactly the same manner as in giving antimeningitic serum. To insure its thorough dissemination throughout the spinal meninges the antitoxin should be diluted, if necessary, with normal salt solution. As a rule, the amount injected should be slightly less than the amount of fluid withdrawn. In the case of a "dry tap," if the operator is reasonably sure of having entered the canal, from 3 to 5 c.c. of serum may be injected. It is generally necessary to repeat this injection within twenty-four hours.

The reason for administering antitoxin subdurally is apparent when it is remembered that neither the central nervous system nor the peripheral nerves take up antitoxin direct from the blood (Park). Only after very large intravenous doses are traces of antitoxin found in the cerebrospinal fluid, and animals passively and actively immunized may be rendered tetanic if the toxin is injected directly into the central nervous system or into the nerve. While antitoxin injected subdurally finally passes over into the blood, it will neutralize any free or dissociated toxin before the latter has developed any harmful tendency.

5. To neutralize any toxin that may have been absorbed by a nerve it may be advisable to inject antitoxin directly into the nerve, and these *intranural injections* under anesthesia are advised by Ashhurst, John, and others as part of the rational treatment of tetanus.

Indications for Treatment.—Every patient, and especially with a history of wound, who presents the symptoms of a stiff neck and rigidity of the masseter muscles on attempting to open the mouth, general muscular rigidity, and especially of the recti muscles of the abdomen causing a board-like resistance to the touch, coming on suddenly and not otherwise definitely accounted for, should receive immediate intraspinal and intravenous injections of tetanus antitoxin, as well as appropriate surgical and general treatment.

General Treatment.—A large number of substances have been advocated in the treatment of tetanus; of these, the most common are injections of phenol and intraspinal injections of magnesium sulphate. While phenol may be well tolerated by tetanus patients, Ashhurst and John believe that

¹ Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1913, xxvii, 1.

² Jour. Amer. Med. Assoc., 1914, lxiii, 235.

all these treatments are of little value, and that spinal injections of magnesium sulphate are dangerous.

1. Chloral hydrate and potassium bromid should be given by mouth or by rectum, in sufficient quantities to produce sleep and quiet. Drugs, such as those of antagonistic physiologic activity, are more or less successful and frequently of aid when given in conjunction with the antitoxin.

Stone¹ has recommended for these purposes the oral or rectal administration every three to six hours of 3 to 5 grains of phenol-barbital (luminal); or chlorbutanol in dose of 15 to 30 grains in diluted alcohol or whisky every three to six hours. Morphin, $\frac{1}{4}$ grain, with atropin, 1/100 grain, may be necessary, but Nicoll² states that conservatism in the administration of depressing sedatives is extremely advisable. The patient should be spared from every possible source of irritation, from noise, bright light, and fussy attention.

2. While combating the disease, the general care of the patient should not be forgotten. A purgative should be administered early. Simple, nourishing, non-stimulating food should be given by the mouth, if possible, or by the nasal tube, if necessary. Absolute quiet should be maintained. Distention of the bladder from retention of urine should be guarded against. If water is not well absorbed, and especially if there is peritoneal or pelvic inflammation, saline injections into the colon should be given.

Surgical Treatment.—1. The site of the wound should be located, and if possible incised under ether or chloroform anesthesia and thoroughly cleansed of foreign material and necrotic tissue. It should then be swabbed with the 3 per cent. alcoholic solution of iodine, washed with hydrogen dioxide solution, and packed loosely with gauze soaked in the iodine solution.

2. Cauterization with pure phenol, followed by alcohol, may be employed, but, as a rule, the weaker germicide is preferable in order not to produce necrosis of the tissues, which furnishes pabulum for bacterial growth. The wound should be dressed daily.

The technic of these injections has been described in Chapter XXXVII.

Serum Treatment; Administration of Antitoxin.—1. Administer 1 c.c. of antitoxin subcutaneously, and if no anaphylactic symptoms occur, give *intravenously* from 10,000 to 20,000 units of antitoxin one hour later, and repeat the dose if no effect is apparent or if the good effect wears off in about six to eight hours (the technic is described in p. 845). Give similar intravenous injections on the second and third days, and the good effects may be maintained by direct intramuscular injections of from 5000 to 10,000 units for one or two additional doses on subsequent days.

2. From 10,000 to 12,000 units should be given *intraspinally* by means of lumbar puncture. This dose should be repeated every eight hours for three or four doses unless the symptoms have markedly ameliorated. The technic of this injection is described on p. 850. A quantity of cerebrospinal fluid should be removed before the serum is injected. After the first injection the fluid may be found to have become cloudy, with a large increase of cells, especially of the polynuclear variety, although bacteriologically it may be sterile. This outpouring of leukocytes is probably a reaction to the irritant effect of the serum, and especially of the preservative it contains.

The intraspinal injections are given under light chloroform anesthesia if necessary.

Children require almost as much antitoxin as adults. Intraspinal injections should never be omitted, although in young children intravenous

¹ Jour. Amer. Med. Assoc., 1922, 78, 1939.

² Jour. Amer. Med. Assoc., 1921, 76, 112.

injections may not be possible, in which case intraspinal and intramuscular injections are given. In young infants serum may be injected intravenously through the longitudinal sinus.

Treatment of the average cases of tetanus in persons twelve or more years old will require from 120,000 to 200,000 units of antitoxin as follows:

First day: 10,000 units intraspinally and 20,000 units intravenously. Repeat both injections eight hours later.

Second day: 10,000 units intraspinally and 20,000 units intravenously. Repeat both injections eight hours later.

Third day: 10,000 units intravenously. If necessary 10,000 units intraspinally.

Fourth day: 10,000 units intramuscularly.

In some cases the first day treatment with antitoxin is sufficient; with others, in which treatment is delayed, more antitoxin may be required.

Results of the Antitoxin Treatment of Tetanus.—As was previously stated, the *prophylactic value of tetanus antitoxin has been proved beyond any reasonable doubt*. This does not imply, however, that the simple introduction of 1000 units of antitoxin beneath the skin will surely protect the patient, as the percentage of cases developing tetanus even after the serum has been given is altogether too high. As was pointed out under Prophylactic Treatment, the wound must receive thorough surgical attention, and the antitoxin must be injected in such places where it will have the greatest opportunity to neutralize the toxin. Even if tetanus should develop under these conditions, it is likely to be mild and the prognosis would be much more favorable.

Tetanus antitoxin has likewise been very successful in veterinary practice, especially after castration and other operations, in injuries, and among horses used for the purpose of producing diphtheria antitoxin and other immune serums.

While the *curative value of tetanus antitoxin has not come up to expectations, more recent carefully prepared statistics indicate that, with serum treatment, the mortality is reduced at least 20 per cent.* as shown by an investigation made by Irons.¹ This includes cases treated by the subcutaneous injection of antitoxin, and it must be emphasized that, in order to secure the best results, tetanus must be treated in a rational manner according to its pathology. Under these circumstances we can confidently expect a greater reduction in mortality. But at any rate no physician should withhold antitoxin in the treatment if there are any possible means of obtaining it. If only 3000 units may be had, it is far better to inject this amount intraspinally than subcutaneously.

Permin,² in a thorough review, reaches the general conclusion that antitetanus serum has reduced the mortality of tetanus approximately 20 per cent. He gives figures from Denmark that are especially valuable, because they were gathered from a small area, and hence represent fairly uniform conditions: Of 199 cases not receiving serum, only 21 per cent. recovered; whereas of 189 cases treated with serum, 42.3 per cent. recovered. Of 92 acute cases with an incubation period of less than ten days 24.2 per cent. recovered when serum was used, whereas of 94 cases treated without serum only 5.3 per cent. recovered. It is significant that these Danish figures correspond closely to the American statistics and those of other countries. Irons³ has recently tabulated the results of 225 cases of tetanus

¹ Jour. Infect. Dis., 1914, 15, 367.

² Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1913, xxvii.

³ Jour. Amer. Med. Assoc., 1914, lxii, 20, 25.

treated with antitoxin collected from hospitals and private records for the years 1907 to 1913. The mortality in all cases receiving serum was 61.77 per cent.; in 21 cases without serum the mortality was 85.7 per cent. The latter figures correspond quite closely with the general mortality of about 85 per cent. of tetanus treated without serum. Irons' figures also show the influence of large doses of antitoxin; of 57 cases receiving a small dose of antitoxin (3000 units or less subcutaneously), the mortality was 73.7 per cent.; of 143 cases receiving large doses (over 3000 units subcutaneously, or 3000 or less intraspinally or intravenously), the mortality was 57.3 per cent. Magnesium sulphate was given intraspinally in 18 cases which also received serum; in 4 cases—2 acute and 2 chronic—the patients recovered, giving a mortality for the group of 77 per cent. In 2 cases death occurred shortly after injection with symptoms of respiratory failure.

The greater the interval elapsing between the onset of symptoms and the time treatment is begun, the greater the mortality.

Likewise whether or not a prophylactic injection of antitoxin was given at the time of injury, has considerable influence on the outcome. For example, in the British Army during the recent war, the mortality was 34.8 per cent. among those developing tetanus after a prophylactic injection of antitoxin and 71.2 per cent. among those not immunized.

The virulence of tetanus infection apparently varies from year to year and modifies the mortality rates. Stone,¹ for example, reports that in the Los Angeles County Hospital the mortality during four years from 1916 to 1921 varied from 14.3 to 71 per cent. with the same general plan of treatment.

All recent writers on the treatment of tetanus have emphasized the value of large doses of antitoxin by intravenous and intraspinal injection, as Fitzgerald,² Ross and Stirrett,³ Bruce,⁴ Golla,⁵ Andrewes and Horder,⁶ Gibson,⁷ Bacti,⁸ Robertson,⁹ Woolf,¹⁰ and others.

In view of this evidence in favor of antitoxin in the treatment of tetanus it is apparent that the physician is compelled to give every patient with tetanus the opportunity to obtain this 20 per cent. or more benefit by administering the serum promptly and correctly.

Non-specific Treatment of Tetanus.—Lüdke¹¹ reports that the treatment of 7 cases of acute tetanus with intravenous injections of 3 to 5 c.c. of a 10 per cent. solution of deuterio-albumose at intervals of twenty-four to forty-eight hours, resulted in greatly diminishing the intensity of the spasms and recovery of all. Kaznelson¹² treated 2 cases in a similar way with albumose injections, with 1 recovery.

SERUM TREATMENT OF GAS GANGRENE

The bacteriology of gas gangrene and prophylactic value of antigas gangrene serum, have been discussed in Chapter XXXVIII. During the Great War a large number of these infections occurred resulting in considerable investigation in reference to etiology and treatment. At the close of the war it was the consensus of opinion that an efficient means of serum prophylaxis and treatment had been finally worked out.

Surgical treatment consisting of thorough cleansing and drainage of the wound to remove the favorable soil for bacteria and particularly the

¹ Jour. Amer. Med. Assoc., 1922, 78, 1939.

² Brit. Jour. Surg., 1916, 4, 14.

³ Canad. Med. Assoc. Jour., 1915, 5, No. 4.

⁴ Lancet, 1917, 1, 680.

⁵ Lancet, 1917, 1, 686.

⁶ Lancet, 1917, 1, 682.

⁷ Amer. Jour. Med. Sci., 1916, 152, 781.

⁸ Bull. d. l'Acad. d. méd., 1916, 86, 316.

⁹ Amer. Jour. Med. Sci., 1916, 151, 781.

¹⁰ Jour. Amer. Med. Assoc., 1922, 72, 1266.

¹¹ Berl. klin. Wchn., 1920, lvii, 344.

¹² Therap. Monatsh., 1917, 31, 437.

anaërobes, and to encourage the inflow of fresh lymph and facilitate phagocytosis, is of maximum importance.

The serum should be polyvalent, that is, prepared by the immunization of horses not only with the toxins of *Bacillus welchii* (*B. perfringens*) but with *B. edemaciens* and *Vibrio septique* as well.

The serum should be given as *early as possible* and before profound toxemia has developed. The administration should be by intravenous injection of 50 to 100 c.c. diluted with saline solution and repeated every six to eight hours until three or four doses have been given. Serum may also be injected in the tissues about the wound in order to effect neutralization of the toxins at the site of production.

Bull,¹ Vincent,² McGlannan,³ Bouchet,⁴ Gibbon,⁵ Van Beuron,⁶ and others have rendered favorable reports, although available statistics are yet too meagre to warrant further statements at this time. The polyvalent serum would appear to be worthy of thorough trial in the treatment of this highly fatal disease.

TREATMENT OF BACILLARY DYSENTERY

Serum Treatment of Bacillary Dysentery.—Soon after the discovery of a bacillus of dysentery by Shiga in 1892 the treatment of bacillary dysentery by the use of immune serums was undertaken. Following the discovery of the etiologic importance of Shiga's bacillus in the dysenteries of Asiatic countries, similar investigations were made in various parts of the world and various bacilli were isolated. At first these micro-organisms were all regarded as being identical, but further investigation has shown that marked differences are apparent, and two main types are now recognized: one type (Shiga) does not ferment mannite and produces a soluble or extracellular toxin, and a second type (Flexner-Harris, Hiss, etc.) ferments mannite and does not produce an extracellular toxin. A further discussion of these bacilli will be found in Chapter VII.

Antidysentery Sera.—The Shiga dysentery bacillus produces a soluble or exogenous toxin and the antiserum is largely antitoxic. This serum is ineffectual for dysentery caused by the Flexner group of bacilli.

The Flexner bacillus produces largely an endotoxin; its antiserum is largely bactericidal and is ineffectual against Shiga infections.

A polyvalent serum may be prepared by mixing these or by immunizing horses with both strains and toxins, as is the usual custom.

Dysentery caused by the bacilli of the Kruse-Shiga type may be regarded as a form of intoxication analogous to the intoxication of diphtheria. The intestine, where the bacilli lodge, corresponds to the throat, which is the site of infection in diphtheria; here the bacilli develop and produce their toxins, and these toxins, when absorbed into the circulation, in turn produce the symptoms of the disease.

Antitoxin has been prepared for the bacilli of the Kruse-Shiga type, and these have yielded fairly satisfactory results in the prophylaxis and cure of this variety of bacillary dysentery. Antiserums for the mannite fermenting group of bacilli (Flexner, Harris, Hiss, Duval, etc.) have not proved of as much value in the treatment of these infections. Bacilli of the latter group are largely responsible for the dysenteries in this country, and also for a percentage of cases of ileocolitis of infancy. Since the antiserum of the Shiga bacillus is of practically no value in the treatment of infections caused

¹ Brit. Med. Jour., 1918, 1, 62.

² Lancet, 1918, 2, 178.

³ Amer. Jour. Med. Sci., 1919, 157, 764.

⁴ Jour. Amer. Med. Assoc., 1919, 73, 239 (includes an excellent review of the subject).

⁵ Surg., Gyn., and Obst., 1918, 26, 336.

⁶ Bull. d. l'Acad. d. méd., 1919, 81, 556.

by bacilli of other groups, the serum treatment of dysentery is employed mainly in European and Asiatic countries, where infections with this group are common. After fairly extensive trials in this country the serum treatment of infantile diarrheas and true dysenteries has proved disappointing.

The *preparation* and *standardization* of *dysentery antitoxin* is described in Chapter XIII.

Administration and Uses.—Dysentery antitoxin has been used both in the prophylaxis and in the cure of this infection. The doses of serum advised by various observers vary considerably, owing to the marked differences that exist in the potency of these serums. Since the various manufacturers do not employ the same standards, the physician should use the serum in accordance with the printed directions that accompany each package.

For *curative* purposes, Shiga has advised 10 c.c. for mild cases and from 20 to 60 c.c. for severer cases. It may be necessary to repeat the injections several times. Vaillard and Doyle have given as much as from 80 to 100 c.c., and have repeated this dose on the following days. When the serum is being used during an epidemic, *it is advisable to ascertain beforehand the nature of the infection, as the antiserum for the Shiga bacillus is highly specific and is not likely to prove of value in the treatment of infections caused by the Flexner type of bacillus.* Otherwise a polyvalent serum should be used.

The injections have usually been given subcutaneously. Better results would, no doubt, be obtained in the treatment of severe infections by administering large doses of serum intravenously.

Flexner¹ has stated "that in cases of ordinary severity, a single subcutaneous dose may be followed by such marked alleviation of the symptoms as not to call for repetition. In other and severer cases the dose may need to be repeated in from twelve to twenty-four hours, and again in forty-eight hours.

"The effects of the injection of the serum tend to appear promptly. They consist first in the amelioration, often within a few hours, of the nervous symptoms and the general prostration. Usually within twenty-four hours the tenesmus and colic disappear and the stools become markedly reduced in number. Along with this improvement there goes diminution in the blood and mucus content of the discharges, coincident with which there is a return of their feculent character. According to the severity of the attack, the stools return to normal in from two to five days.

"Acute cases of bacillary dysentery are especially subject to the serum treatment, but cases in their second to third week may still be favorably influenced, as may also relapses in the course of convalescence from acute attacks. On the other hand, chronic cases and the recrudescences in the course of chronic dysentery, even if originally induced by the dysentery bacilli, do not as a rule respond to the serum.

"It should be added that the employment of the serum does not contraindicate the usual eliminative and dietetic forms of treatment."

Results.—Adequate statistics regarding the value of the serum in the prophylaxis and treatment of dysentery are favorable in so far as Shiga infections are concerned. From the *prophylactic* standpoint, encouraging results have been reported by Kruse, Shiga, Vaillard and Dopter, Rosculet, and others, and it would appear that passive immunization is of value in combating localized outbreaks, such as occur in institutions and armies.

From the *curative* standpoint, most observers agree that the use of a potent serum will reduce the mortality of acute cases at least from 30 to

¹ Jour. Amer. Med. Assoc., 1921, 76, 108.

50 per cent. The mortality has varied widely from 5 to 50 per cent. in different outbreaks and epidemics. As a general rule the Shiga infections are more severe than the Flexner infections, but this relation may be reversed. Flexner states that in mild outbreaks with a mortality of 10 to 15 per cent., serum treatment may reduce this rate to 1 to 2 per cent. In severer epidemics serum treatment may reduce the mortality from 50 per cent. to about 10 per cent. Shiga reports a drop in the mortality in Japan of from 22 to 26 per cent. to 9 to 12 per cent.; Kruse obtained a reduction in mortality of about 10 per cent. Vaillard and Dopfer¹ treated 96 cases, with but 1 death; Rosenthal² treated 157 cases with 7 deaths—a mortality of 4.5 per cent. as compared with that of 10 to 11 per cent. occurring in other German hospitals. Coyne and Auché³ treated 11 cases due to the Flexner type of bacillus and report good results. Ruffer and Willmore,⁴ Violle,⁵ Rogers,⁶ Brau,⁷ and Baht⁸ have also reported favorably upon the use of the serum.

During the recent Great War bacillary dysentery proved a frequent and troublesome infection and particularly in 1917–1919. The Flexner infections appeared to predominate. Nolf⁹ reports that serum treatment failed whereas vaccine treatment and particularly by intravenous injection, yielded very good results (see later). Klein,¹⁰ however, reported good results when 60 to 100 c.c. of serum was injected intravenously early in the disease. Finlayson,¹¹ Waller,¹² Pribaum,¹³ Klesk,¹⁴ and others have published generally favorable reports.

A highly potent and polyvalent serum should be in readiness during war, and particularly in camps and hospitals. In a thorough investigation made in the United States in 1903 by the Rockefeller Institute, under the direction of Flexner, it was found that the results of the serum treatment of ileocolitis, among children at least, were quite disappointing. Josephs and Davison¹⁵ for example, found that the intramuscular and subcutaneous injections of from 20 to 50 c.c. of serum did not reduce the mortality or influence the course of the disease in a series of acute dysenteries among 20 children in this country (17 being Flexner and 3 Shiga infections). This is largely due to the fact that several different strains of bacilli may be the cause of an infection, and unless a corresponding antiserum is employed for the particular type causing the infection in a given case, good results cannot be expected. Probably if some means were discovered for making a prompt bacteriologic diagnosis, and if several immune serums were on hand for the treatment of infections caused by the main types, after the methods worked out by Neufeld, Dochez, and Cole in the treatment of pneumonia, better results may be obtained.

¹ *Ann. de l'Inst. Past.*, 1906, xx, 321; 1907, xxi, 241.

² *Deutsch. med. Wchn.*, 1904, xxx, No. 19.

³ *Compt. rend. Soc. de biol.*, 1906, lx, No. 26.

⁴ *Brit. Med. Jour.*, 1908, ii, 1176; *ibid.*, 1909, ii, 462.

⁵ *Arch. de méd. et pharm. nav.*, 1912, xcvi, 61.

⁶ *Dysenteries, Their Differentiation and Treatment*, London, 1913, 290.

⁷ *Ann. d. hyg. et méd.*, 1913, xvi, 710.

⁸ *Brit. Med. Jour.*, 1914.

⁹ *Jour. Amer. Med. Assoc.*, 1919, 73, 1177.

¹⁰ *Lancet*, 1919, 2, 775.

¹¹ *Brit. Med. Jour.*, 1917, 1, 46.

¹² *Lancet*, 1919, 2, 778.

¹³ *Centralb. f. Bakteriöl.*, 1918, 80, 33; *ibid.*, 1918, 81, 37.

¹⁴ *Med. Klin.*, 1915, 11, 1147.

¹⁵ *Jour. Amer. Med. Assoc.*, 1921, 77, 1863. (This paper gives a good bibliography of the serum treatment of dysentery.)

According to Davison¹ the oral and rectal administration of 5 to as much as 1381 c.c. of D'Herelle's *dysentery bacteriolysant* had no influence upon the course or mortality of bacillary dysentery in children. This material is prepared by cultivating a small portion of feces (dysenteric convalescent or normal) in bouillon and filtering through a Berkefeld filter; when the filtrate is added to cultures of dysentery bacilli they are killed and dissolved.

The curative effect of dysentery antitoxin is shown by a reduction in the number of stools, by the fact that blood and pus disappear from the discharge, pain and tenesmus are relieved, the temperature becomes normal, and the patient gains in weight. Individual observers are frequently enthusiastic over the results obtained in individual cases, and no doubt these are striking in those instances where the antiserum appears to be specific for the particular form of infection.

Vaccine Treatment of Bacillary Dysentery.—*Vaccines by Subcutaneous Administration.*—In the very acute choleric types characterized by violent diarrhea and vomiting with rapid dehydration of the tissues, vaccines have not generally been employed; in these the subcutaneous injection of 1 to 2 liters of saline solution and fluid diet has proved a successful plan of treatment.

In subacute ulcerative dysentery due to Flexner bacilli, Nolf and his associates² have reported good results from the subcutaneous injection of heat-killed autogenous vaccines beginning with a dose of 10,000 and gradually increasing to 5,000,000,000 to 10,000,000,000, which were well tolerated by most individuals.

In old chronic cases Nolf³ has likewise reported favorably upon the results of treatment with heated vaccines beginning with a dose of 1,000,000 and increasing until 5,000,000,000 to 10,000,000,000 are being injected at one time. "In every case the general condition was improved, and the intestinal symptoms steadily decreased. In the majority the cure was complete and definite. At times there seemed to be complete cure at the end of treatment, but at a later period the symptoms returned. In some cases the stools, though regular and only one or two a day and without blood and mucus, yet remained soft, and there persisted a little intestinal instability and discomfort."

Vaccines by Intravenous Administration.—Nolf has reported even more favorably upon the results of the intravenous administration of heat-killed vaccines:

"The doses were given at four-day intervals, the initial dose being regularly 10,000 germs, then 30,000, then 50,000, then 100,000, etc. In general, the betterment of the patient did not long delay. The fever dropped by lysis, with some recrudescences more or less marked on the days of the vaccine therapy and the next day; and the intestinal symptoms improved coincidently. In many cases of moderate intensity a complete cure was effected when the dose of 500,000 was reached. In the more refractory cases it was necessary to push the vaccine up to about 10,000,000.

"In 52 cases treated in this way we had only 2 deaths. All the other patients left the hospital cured except 2, whom military necessity forced us to send away too soon. We have no doubt that in these 2 cases also the continuation of the treatment would have resulted in a cure in a relatively short time. By vaccinothrapy we were thus able to avoid the dangerous

¹ Amer. Jour. Dis. Child., 1922, 23, 531.

² Arch. méd. belges, May, 1918.

³ Jour. Amer. Med. Assoc., 1919, 73, 1177.

tendency toward chronicity which in 1917 was produced in a considerable number of our patients. This last result we considered particularly gratifying.

"The complete record of the epidemic of bacillary dysentery of 1918 shows a complete cure, at the latest in a few weeks' time, in 500 cases, except only 2 patients who died, and 2 who left before the cure was completed."

Non-specific Treatment of Bacillary Dysentery.—Doubtless part of the beneficial results observed by Nolf in the treatment of bacillary (Flexner) dysentery with intravenous injections of vaccine are to be attributed to non-specific effects.

Adler¹ and Dollken² have reported favorable results from the intramuscular injection of 3 to 5 c.c. of sterile *milk* and *especially as a means for treating intestinal hemorrhage*; this form of treatment appears very worthy of trial in conjunction with other therapeutic measures.

Dollken, Lüdke, and Holler have also employed intravenous injections of 10 per cent. solution of deutero-albumose in dose of 1 to 2 c.c. for three to six injections in the treatment of dysenteries mostly caused by the Shiga bacillus; of 12 cases treated in this manner by Lüdke, good results were secured in 10.

TREATMENT OF MENINGOCOCCUS MENINGITIS (CEREBROSPINAL FEVER)

Types of Meningococci.—Prior to 1909 meningococcus meningitis was regarded as caused by a single strain of the meningococcus. In 1909 Dopter³ discovered the para-meningococcus, differentiable from the meningococcus by immunologic reactions and especially by the agglutination reaction. In 1915 Gordon and Murray⁴ classified all meningococci into four groups by means of agglutination tests. According to Flexner, Gordon's Type I appears to correspond with the "parameningococcus" of Dopter, and Type II with the normal or regular meningococcus. Types III and IV appear to conform to the more common intermediates. According to Nicolle's classification, his Type A corresponds to Gordon's Types I and III and his Type B to Gordon's Types II and IV. The differentiation is made by agglutination tests, the technic being described in the chapter on Agglutinins. It is very much hoped that an international classification may be adopted.

Worster-Drought and Kennedy⁵ have classified the meningococci from 42 cases according to Gordon's types with these results:

(a) 10, or 23 per cent., due to Type I; all were severe infections, 6 proving fatal.

(b) 20, or 49 per cent., due to Type II; this coccus was most frequently met with both in carriers and in actual cases; 14 recovered and 6 died.

(c) 10, or 23 per cent., due to Type III; 6 recovered and 4 died.

(d) 2, or 5 per cent., due to Type IV; 1 case died.

In a general way it would appear that Types I and III are more virulent as regards the meninges than Types II and IV, but both Rolleston⁶ and Adshead⁷ believe that observations on a larger scale are required before any

¹ Wien. med. Wchn., 1917, 67, 509.

² Münch. med. Wchn., 1919, 66, 480.

³ Compt. rend. Soc. de biol., 1909, 66, 772, 1055; *ibid.*, 1909, 67, 74; *ibid.*, 1909, 69, 600.

⁴ Jour. Roy. Army Med. Corp., 1915, 25, 410, 456.

⁵ Cerebrospinal Fever, A. & C. Black, London, 1919, 270.

⁶ Lancet, 1919, 1, 541.

⁷ Med. Research Committee, Special Report Series, No. 17.

statement can be made on the possible relationship of these strains to the severity of the disease.

The Mechanism of Infection of the Meninges.—The path by which meningococci reach the meninges has been thought to be (a) direct invasion of the cerebral meninges by way of the lymphatics from the nasopharynx and accessory nasal sinuses; (b) infection along the lymphatics of the spinal nerve roots; and (c) primary invasion of the blood-stream from the nasopharynx with secondary localization of the meningococci in the meninges.

Westenhoeffer¹ advanced the theory that infection took place through the sphenoidal sinuses and Netter and Debre through the ethmoid sinuses. Elser and Huntoon,² Worster-Drought and Kennedy,³ and others have been unable, however, to find any evidence in support of the sphenoid pathway, and the latter point out that when sphenoid suppuration occurs it may be secondary.

Flexner⁴ in 1917 considered that meningococci probably passed along the lymphatics around the olfactory nerves to the meninges, but Austrian's⁵ experiments yielded negative results.

The theory that infection may occur along the lymphatics of spinal nerves with the primary foci of meningococcus infection in the intestines, lungs, or cervical glands, was put forward particularly by McDonald,⁶ but has since been discredited by Symmers⁷ and others, and now commands but little attention.

Meningococcus Bacteremia.—Herrick⁸ has recently called particular attention to the occurrence of meningococci in the blood-stream and that by special cultural methods they may be found in 50 to 80 per cent. of cases examined at an early stage and frequently before meningitic symptoms are well developed. In some cases the bacteremia may apparently exist without the development of meningitis. Baeslack⁹ obtained positive results in 36 per cent. of early cases, but the general average of positive blood-cultures is about 25 per cent. Bloedorn¹⁰ gives a good review of the literature and reports a case.

There cannot be any doubt that meningococcus bacteremia occurs in many cases, although it tends to be intermittent and temporary and may require a series of blood-cultures for its detection. This blood infection, however, is important in relation to serum therapy as well as in relation to the mechanism of infection of the meninges.

Elser and Huntoon, Austrian and Herrick believe that meningitis is produced first by blood invasion with secondary involvement of the meninges by way of the choroid plexes which either filters out the cocci and is first infected or by selective localization of the cocci in the pia mater.

It is probable that the pathway of infection varies in different cases. As Huntoon and Elser have pointed out infection may occur by extension from the ear, mastoid, or sinus infection when the onset is gradual, just as pneumococcus meningitis is so frequently produced. In the majority of instances, however, and especially in the epidemic form of the disease with sudden onset, infection appears to take place by passage of meningococci

¹ Berl. klin. Wchn., 1905, 62, 737.

² Jour. Med. Research, 1909, 20, 517.

³ Lancet, 1917, 2, 711.

⁴ Jour. Amer. Med. Assoc., 1917, 69, 639.

⁵ Bull. Johns Hopkins Hosp., 1918, 29, 183.

⁶ Rev. Neurol. and Psych., Edinb., 1907, 5, 702.

⁷ Lancet, 1908, 2, 472.

⁸ Arch. Int. Med., 1918, 21, 541; Jour. Amer. Med. Assoc., 1918, 71, 612.

⁹ Jour. Amer. Med. Assoc., 1918, 70, 684. ¹⁰ Amer. Jour. Med. Sci., 1921, 172, 881.

from the nasopharynx into the lymphatic and vascular channels with invasion of the blood-stream and localization in the meninges. As stated by Bloedorn, "it would appear that cases of meningococcus septicemia are becoming more frequent, or at least more frequently recognized, and that it is well to be on the alert for such cases in order that the diagnosis may be made and treatment instituted before the development of meningitis or endocarditis. The appearance in an acute febrile case of petechiæ or a maculopapular eruption resembling at times the rose spots of typhoid fever, together with a moderate leukocytosis and a temperature chart, which may either be of the septic type or may resemble markedly the chart of malarial fever, should rouse the suspicion of meningococcus sepsis and should lead to repeated blood-cultures being made in an effort to identify the meningococcus. The skin lesions of meningococcus sepsis are particularly striking. The eruption may be purpuric, hemorrhagic, or maculopapular. In the acute fulminating cases, which usually die within the first few days, a purpuric generalized eruption is the rule. These cases are quickly overwhelmed and die frequently before the development of meningitic symptoms. In the protracted cases of meningococcus sepsis the character of eruption shows more of a tendency to assume the maculopapular or hyperemic type."

The main symptoms and lesions of the disease, and several of the complications, for example, the paralyses, eye complications, deafness, hydrocephalus, and mental disturbances, are probably directly due to *suppuration of the meninges*, with involvement of accessory and motor nerve-roots, meningeal irritation, and pressure from the accumulation of exudate in the ventricles and subarachnoid space. Complications, such as arthritis, pyelitis, endocarditis, adenitis, etc., are due to the *bacteremia*, which may become chronic and be accompanied by deposits of meningococci in the various tissues and organs. In addition to these complications there is probably a varying degree of general *toxemia*, due to a soluble toxin or endotoxin liberated through lysis of the cocci.

Although cerebrospinal meningitis may be considered primarily as a general infection, in the majority of instances local suppuration of the meninges constitutes the main lesion. For anatomic and physiologic reasons, however, it is impossible to treat the disease according to the ordinary principles governing the treatment of localized suppuration, as, for example, by continuous drainage and by cleansing the affected parts with germicidal solutions. Unaided, the leukocytes and body fluids are generally unable to destroy the cocci and terminate the infection before serious harm to important nerves and nerve-centers has resulted, so that epidemic meningitis, with a mortality of from 75 to 90 per cent., and followed by more or less serious sequelæ, which few survive, is one of the most dreaded of infections.

In administering antimeningitic serum we aim to assist the patient's leukocytes and body fluids to overcome the infection. Repeated spinal punctures remove portions of the infective material mechanically, but the greatest dependence in bringing about quick destruction of the cocci and effecting recovery of the patient is to be placed upon the serum.

The Serum Treatment of Meningococcus Meningitis.—*Historical.*—During the pandemic of meningococcal cerebrospinal meningitis in 1904-05 several laboratories sought to produce an immune serum for the purpose of treating human cases of this infection.

After pursuing experimental studies on the subject on the lower animals, Jochmann,¹ in 1905, immunized a horse and used the serum in the treatment

¹ Deutsch. med. Wchn., 1906, xxii, No. 20, 788.

of 38 cases of epidemic meningitis. At first he employed the subcutaneous method of injection, and later he used the intraspinal method. The results were quite encouraging, and during the following year 30 more cases were treated, with a resulting mortality of 27 per cent., as against a mortality of 53 per cent. in untreated cases.

At about the same time Kolle and Wassermann¹ reported that they had prepared an antimeningococcus serum, which had not, however, up to that time been used in the treatment of human infections. A year later serum was administered subcutaneously and then intraspinally, with encouraging results.

In this country Park had, in 1905, prepared an antimeningococcus serum, which was used in the treatment of 20 cases in Hartford, Conn., by subcutaneous injection, but without beneficial results. Jochmann had, in the mean time, shown the superiority of intraspinal injections, and this method soon supplanted the subcutaneous method.

In 1905 Flexner began a series of studies regarding experimental meningococcus infections in the lower animals, and the therapeutic value of antimeningococcus serum. These valuable experiments attracted the attention of the world, and placed this method of treatment upon a firm basis. In 1906 Flexner² proved that a specific immune antimeningococcus serum could be produced that, if injected intraspinally, would save the lives of monkeys. Later horses were immunized and the serum used in the treatment of human infections during an epidemic in Akron, Ohio, in May, 1907. In a short time the serum was used extensively in other epidemics, and a report of these early cases was made by Flexner³ in 1907. A later report by Flexner and Jobling,⁴ covering the treatment of 400 cases, showed that the mortality had been reduced from 75 per cent. to below 30 per cent. In 1909 they reported⁵ upon 712 cases treated with the serum, with a mortality of 31.4 per cent. In a more recent report Flexner⁶ reviewed all the cases—1300 in number—gathered from all parts of the world, treated with serum prepared in the Rockefeller Institute. The general mortality rate is given as 30.9 per cent., as against 75 to 80 per cent. among cases not receiving serum treatment. Of 1394 cases treated with serum during the Texas epidemic⁷ (1912), the mortality was 37 per cent., as compared with a mortality of 77 per cent. among 562 cases receiving no serum.

Good results have been reported by many observers with Jochmann's, Kolle and Wassermann's, Ruppel's, Paltauf's, and Dopfer's serums, and the serums have been prepared by several commercial biologic laboratories, so that the curative value of antimeningococcus serum is definitely established.

Preparation of the Antimeningococcus Serum.—Method of Flexner and Jobling.—

1. Many strains of meningococcus are used in order that a polyvalent serum may be prepared. Fresh strains from new epidemics and sporadic cases are constantly added. "*Fast*" strains, or those isolated from cases in which the serum has produced no beneficial effect, are especially desirable. For polyvalent sera the more strains employed, the better the serum; at the Rockefeller Institute as many as 50 different strains have been employed. As shown by Amoss, Gates and Olitsky,⁸ horses immunized with five strains including the different types of meningococci, produce antibodies for the various strains, but these sera do not keep

¹ Deutsch. med. Wchn., 1906, xxxii, No. 16.

² Jour. Amer. Med. Assoc., 1906, xlvii, 560.

³ Jour. Exper. Med., 1907, ix, 168.

⁴ Jour. Exper. Med., 1908, x, 141.

⁵ Jour. Amer. Med. Assoc., 1909, liii, 1443.

⁶ Jour. Exper. Med., 1913, xvii, 553.

⁷ Sophian: Epidemic Cerebrospinal Meningitis, St. Louis, 1913. Report of Dr. Steiner, President of the Texas State Board of Health.

⁸ Jour. Exper. Med., 1920, 32, 767.

as well or prove ultimately as therapeutically active as sera prepared by immunizing with a large number of strains. Wadsworth,¹ however, has recently reported that by immunization with a limited number of representative strains, four or six, carefully selected on account of their antigenic and agglutination properties, the potency was increased three- to tenfold without sacrificing the polyvalency; that is, as tested with at least 70 heterologous strains of the meningococcus.

2. Immunization is performed first with an autolysate of the meningococci, and later with living cultures.

3. Stock cultures are kept alive by transplanting them every four days in slants of ascitic glucose agar, neutral to phenolphthalein.

4. In preparing the autolysate, the cultures are subcultured first on glucose-agar slants without serum. After twenty-four hours' growth about 3 c.c. of salt solution are added to each slant, and the culture emulsified. One c.c. is then poured over the surface of glucose-agar slants in large 500 c.c. Blake bottles. After twenty-four hours' incubation heavy uniform, and diffuse growths are secured.

Add 10 c.c. of normal salt solution to each bottle and wash off the culture. If necessary a long heavy platinum loop may be used. Each bottle is tested for contamination by staining a smear according to the method of Gram. Each bottle is emptied into a common vessel; 2 per cent. toluol is added, mixed well, and incubated for from eighteen to twenty-four hours. The toluol is then allowed to evaporate, or it may be immediately filtered off through sterile gauze saturated with salt solution. The preparation is kept in a refrigerator and should be prepared fresh every month.

5. The *injections* are given subcutaneously *about the neck and abdomen*. Young and healthy horses are selected for the purpose. The first dose consists of 2 c.c. of the autolysate, and this is gradually increased, depending on the manner in which the animal reacts, until 10 c.c. are given in a single dose. Then inject 2 c.c. of living culture diluted with 2 parts of salt solution, and increase the doses, the same as with the autolysate, until 10 c.c. of culture are given at one injection. Next inject living cultures and autolysate alternately, until a maximum of from 30 to 35 c.c. are given in one dose; this last is then used as the constant dose until the immunization has been completed.

Injections are given every five to seven days until the large doses are reached, when they are given every ten days or two weeks.

Horses usually show quite marked reactions, such as fever, depression, and induration about the site of injection, but if due care is exercised, few animals are lost during the immunization.

6. *Bleedings* are usually begun about the fourth month after immunization has been instituted. The horses are bled aseptically from a jugular vein about every two weeks, from 8 to 8 liters of blood being removed at each sitting. The serum is separated and preserved with trikresol. *Recent investigations indicate that trikresol may be partly responsible for paralysis of the respiratory centers, and at present every effort should be made to collect and market the serum in a strictly aseptic manner, so that none or but very little preservative is required.*² Efforts are being made at present to discover an efficient volatile antiseptic that may be driven off by warming the serum at body temperature.

*Rapid Method of Amoss and Wollstein.*³—These investigators have recently advocated a rapid method of immunization consisting in inoculating alternately several strains of living meningococci and parameningococci and the autolyzed products of each, by which a potent polyvalent serum may be produced in eight to twelve weeks instead of in the ten months required by the subcutaneous method. In the preparation of a polyvalent serum a twenty-four-hour agar slant culture of meningococci is removed with 2 c.c. of salt solution and 0.1 c.c. suspended in 15 c.c. of salt solution and injected very slowly into the circulation of a horse. The temperature is taken hourly and should not rise over 3° C.; twenty-four hours later 0.2 c.c. of suspension, and on the third day 0.3 c.c. are given. After the lapse of seven days a series of three injections of living parameningococci are given in doses of about 0.3 c.c. of the emulsion or sufficient to give a temperature reaction of about 2.5° to 3° C. After a lapse of seven days three intravenous injections of an autolysate composed of equal parts of autolysate of meningococci and parameningococci are given. A series of three injections of living meningococci and parameningococci follow in doses which may be run up to 0.6 c.c. of emulsion, with every third series consisting of injections of the mixed autolysates; after ten to twelve weeks a potent serum is generally produced. In order to make the serum as polyvalent as possible a large number of strains of meningococci and parameningococci should be used.

Severe reactions due to hypersensitiveness of the horse to the meningococci or its products are especially likely in the first dose of each series after three or four series of injections have been given. In order to desensitize, a portion of the first injections of each series is

¹ Jour. Exper. Med., 1921, 33, 107.

² Hall, W.: Bull. No. 91, Hyg. Lab., U. S. P. H. S., 1914.

³ Jour. Exper. Med., 1916, 23, 403.

injected intravenously, and two hours later the remainder of the dose is given. Danger due to agglutinated cocci is lessened by diluting the dose in 15 to 20 c.c. of salt solution and injecting very slowly.

The Preservation of Antimeningococcus Serum.—The subject of proper preservation of antimeningococcus serum is one of considerable importance by reason of the untoward effects that may be produced when the serum is injected intraspinally. Ordinarily 0.2 to 0.3 per cent. tricresol is employed. Kramer,¹ however, has declared that tricresolized serum is dangerous. Hale² has shown that tricresol injected intraspinally in dogs may produce paralysis of the respiratory center, while Auer³ showed that the respiratory changes were much less marked in monkeys and due to a stimulation of the inhibitory function.

Flexner⁴ and Fitzpatrick, Atkinson and Zingher⁵ have attributed untoward effects in human beings to the pressure exerted by intraspinal injections of serum rather than to the presence of tricresol.

Chloroform has also been employed as a preservative, but as reported by Neal and Abramson⁶ the serum produces so much pain when injected that this method is objectionable; furthermore, chloroform is much less bactericidal than tricresol.

Tricresol, therefore, is being usually employed at the present time in 0.2 to 0.3 per cent. Both Hale² and Leake and Corbitt⁸ have found tricresol and phenol of about equal toxicity, the M. L. D. for mice being approximately 0.00037 gram per gram weight of mouse. Tricresol, however, is more bactericidal.

Krumwiede and Banzhaf⁹ have advised using equal parts of cresol and ether as a means for preventing the immediate precipitation of the serum proteins and clouding of the serum, although subsequent precipitation may occur. Masucci¹⁰ found that the mixture of ether and cresol had no advantage as a preservative of serum over straight ether in the total precipitate formed on standing or in germicidal value.

Standardization of Antimeningococcus Serum.—An accurate method of standardizing antimeningococcus serum has not as yet been devised. In the selection of a serum physicians must, therefore, be guided by the reputation of the manufacturers.

The main point at issue concerns the nature of antimeningococcus serum, that is, the mechanism of its curative action and the chief antibodies concerned in the process. According to Jochmann,¹¹ Flexner,¹² Flexner and Amoss,¹³ Evans,¹⁴ and others the chief activity of the serum is by increasing phagocytosis of the meningococci; Flexner also thought that the serum may be antiendotoxic, and Flexner and Jobling¹⁵ have found that potent sera may directly injure the organisms and impair their power of propagation.

Five different methods have been advocated for testing the potency of these sera: (a) By determining the opsonin or tropin content as originally advocated by Jobling¹⁶ and regarded at present by the Hygienic Laboratory as the method of choice. Certainly in the treatment of meningococcus meningitis one is impressed by the evidences of increased phagocytosis of the cocci in cases favorably influenced by the serum, and this test appears to the writer as being a direct measure of at least one of the important curative activities. (b) By determining the agglutinin content as described by Amoss and Wollstein,¹⁷ Wadsworth, Kirkbride, and Gilbert.¹⁸ (c) By determining the complement-fixation titer as advocated by Krumbein and Schatloff¹⁹ and widely employed in Germany. (d) By determining the protective power of the serum in mice infected with virulent cultures as advocated by Hitchens and Robinson.²⁰

1. *Bacteriotropic Titration.*—While the antimeningitic serum was being prepared at the Rockefeller Institute Jobling used the opsonic test in standardization as the method of choice on account of the part taken by specific opsonins in promoting recovery from meningococcus infections. As a definite and suitable standard of strength Jobling has suggested that a serum be accepted as satisfactory when it shows unmistakable phagocytic activity in dilutions up to 1 : 5000. The method of Neufeld is used, as described on page 184.

The method described by Evans as used in the Hygienic Laboratory (Bulletin No. 124) is as follows:

(1) The serum is diluted with Locke's solution 1 : 25, 1 : 50, and 1 : 150; 0.2 c.c. of each

¹ Jour. Amer. Med. Assoc., 1913, 60, 1348.

² Hygienic Laboratory Bulletin, 1913, No. 91.

³ Jour. Amer. Med. Assoc., 1914, 62, 1799; Jour. Exper. Med., 1915, 21, 43.

⁴ Jour. Amer. Med. Assoc., 1913, 60, 1937.

⁵ Collected Studies from Bureau of Laboratories of New York City, 1914-15, 37.

⁶ Jour. Amer. Med. Assoc., 1917, 68, 1035.

⁷ Hygienic Laboratory Bulletin, 1913, No. 88.

⁸ Hygienic Laboratory Bulletin, 1917, No. 110, 35.

⁹ Jour. Infect. Dis., 1921, 28, 367.

¹⁰ Jour. Infect. Dis., 1922, 30, 379.

¹¹ Deutsch. med. Wchn., 1911, 37, 1733.

¹² Jour. Exper. Med., 1907, 9, 168.

¹³ Jour. Exper. Med., 1916, 23, 683.

¹⁴ Hygienic Lab. Bull., 1920, No. 124, 43.

¹⁵ Jour. Exper. Med., 1908, 10, 141.

¹⁶ Jour. Exper. Med., 1909, 11, 614.

¹⁷ Jour. Exper. Med., 1916, 23, 403.

¹⁸ Arch. Int. Med., 1919, 23, 269.

¹⁹ Deutsch. med. Wchn., 1908, 24, 1002.

²⁰ Jour. Immunology, 1916, 1, 345.

dilution are placed in test-tubes measuring 10 x 75 mm. A fourth tube carries 0.2 c.c. of 1 : 25 normal horse-serum, and a fifth, 0.2 c.c. of Locke's solution (control).

(2) Young and rapidly growing cultures of meningococci are employed (thirteen- to twenty-one-hour cultures on serum glucose agar). The suspensions are made by washing off the growths in each tube with 2 c.c. of equal parts of ordinary broth and Locke's solution and diluting until the turbidity equals the standard equivalent to 300 parts per million of silica adopted by the American Public Health Association; 0.2 c.c. of this suspension is added to the tubes carrying the serum dilutions which now gives final dilutions of 1 : 50, 1 : 100, and 1 : 300. These mixtures are placed in a water-bath at 37° C. for forty-five minutes and in the meantime the leukocyte suspension is prepared.

(3) On the preceding day a rabbit is injected intrapleurally on each side with 5 c.c. of a sterile aleuronaut suspension (starch 3 gm.; aleuronaut 5 gm., and ordinary broth 100 c.c.). On the following day the animal is chloroformed and the exudates in both cavities washed out into a 50 c.c. centrifuge tube with 1 per cent. solution of sodium citrate warmed to body temperature. Care is exercised against using bloody exudates. If aleuronaut is removed, the heavy particles soon settle and the supernatant suspension of leukocytes is transferred to a second 50 c.c. tube and centrifuged for four minutes at such speed as throws the leukocytes to the bottom without too much impaction.

The supernatant fluid is discarded, the tube filled up with warm saline solution, the leukocytes gently distributed, and the tube centrifuged. The resulting mass of leukocytes are now resuspended in about 10 c.c. of warm Locke's solution.

Leukocytic suspension (0.2 c.c.) is added to each tube at the end of the forty-five minutes of incubation and the contents gently mixed and reincubated for forty-five minutes. Twice during this period each tube is rolled vigorously between the palms of the hands in order to keep the leukocytes in suspension.

(4) Smears are now rapidly made, dried in the air, fixed with methyl alcohol, dried without washing, stained with a weak solution of carbol toluidin blue for twelve seconds (5 gm. toluidin blue dissolved in 100 c.c. alcohol, 500 c.c. water and 500 c.c. of 5 per cent. phenol, filtering after one or two hours. One part of the stain is diluted with 2 parts of water for staining the smears), and then with a solution of safranin (4 drops of a 0.5 per cent. watery or alcoholic solution of safranin in 50 c.c. of water) for ten minutes or longer.

(5) The controls usually show that 4 to 20 per cent. of the polymorphonuclear leukocytes have engulfed cocci. The highest dilution of the immune serum showing 32 per cent. or at least twice as many phagocytes as the controls expresses the titer. No attempt is made to average the cocci contained in the leukocytes, but simply the highest dilution, producing at least twice as much phagocytosis as observed in the two controls.

2. *Complement-fixation Tests.*—The advantages of these tests are that the same polyvalent antigen may be used as is employed for purposes of immunization; the technic is simple and the reactions are usually sharp and definite. According to Sophian, in a series of comparisons with opsonic and complement-fixation tests the results corresponded in every instance, a high opsonic content being accompanied by a high complement-fixation reading. The latter indicates, at least, that the horse has responded to immunization and that curative antibodies probably are present. Laboratories usually adopt their own standards in preparing antimeningococcic serum. In the complement-fixation test Kolle requires complete inhibition of hemolysis with 0.1 c.c. of serum.

Hitchens and Hansen¹ have advocated a dried meningococcus antigen prepared as follows:

- (1) The cultures are grown on salt-free agar at 37° C. for sixteen to eighteen hours.
- (2) The growth is collected and suspended in distilled water; about 10 c.c. distilled water being used for each 20 square inches of agar surface.
- (3) To this suspension is added an equal volume of 95 per cent. ethyl alcohol.
- (4) This mixture is immediately centrifugalized.
- (5) After the supernatant fluid has been removed the precipitated bacteria are again suspended in 95 per cent. ethyl alcohol.
- (6) This process of centrifugalization and resuspension is repeated a second and a third time, but with ethyl ether instead of alcohol, the original volume of the suspension being reduced one-half each time.
- (7) The ether adhering to the bacteria after the precipitation and after removal of the supernatant ether is removed by vacuum.
- (8) The bacterial mass now constituting the antigen is further dried over phosphorus pentoxid *in vacuo* for three days.
- (9) The antigen is stored in tubes—about 0.05 gram to each—and kept *in vacuo* over phosphorus pentoxid.

For use, a small amount of the powder, 0.02 gram, is carefully ground in a mortar, 20 c.c. of normal salt solution being gradually added.

The technic of these tests is given in Chapter XXV.

¹ Jour. Immunology, 1916, 1, 355.

3. *Agglutination Tests*.—These tests are readily conducted with the polyvalent antigen used in immunization, a macroscopic technic, as that described in Chapter XVI, being employed.

Wadsworth, Kirkbride, and Gilbert have described the following method as employed by the Laboratory of the New York State Department of Health:

(1) Standard cultures of meningococcus representative of the principal types may be obtained from the Laboratory of the State Department of Health. They are cultures which have been identified by serologic tests and which possess distinctive agglutinative and antigenic properties.

(2) Standard suspensions for performing the agglutinating tests are so prepared as to correspond in opacity with a standard suspension of barium sulphate containing 3 c.c. of a 1 per cent. barium chlorid solution in 97 c.c. of a 1 per cent. sulphuric acid solution. Such a standard meningococcus suspension contains approximately 2,000,000,000 cocci per cubic centimeter.

(3) In testing the potency of serum normal saline solution (0.85 per cent.) should be used as the diluent. After dilution with the serum the final mixture should contain 1,000,000,000 cocci. The standard culture suspensions should be added in agglutination tubes (8 to 10 mm. in diameter), to the diluted serums to be tested, and also similar dilutions of the standard serum. These test mixtures should be incubated at 55° C. for from sixteen to twenty-four hours. The titer thus determined is the maximum dilution of the serum in which definite clumping of the meningococcus in the suspension can be easily detected by the unaided eye. The agglutinative titer which is to be recorded is the potency of the serum.

(4) The potency of antimeningococcus serum intended for therapeutic use, when tested and compared with the standard serum supplied on request by the Laboratory of the New York State Department of Health, shall have an agglutinative action in a dilution of 1 : 800 with at least the four representatives of the principal types of meningococcus, under conditions in which a standard suspension is employed and the tubes maintained for sixteen to twenty-four hours at 55° C.

4. *Animal Inoculation Tests*.—These tests have been found quite irregular and impracticable for general use. As stated by Jobling, not only does the pathogenicity of the meningococcus vary considerably from day to day, but the resistance of animals to this micro-organism is also quite variable. By preparing a large quantity of bacterial emulsions and using sufficient controls to determine the fatal dose, and by employing a standard lethal dose of emulsions mixed with varying quantities of serum injected intraperitoneally into 250-gram guinea-pigs, some conception of the protective value of a serum may be obtained.

Hitchens and Robinson¹ have recently described an animal inoculation test which they hoped would prove of value for the standardization of antimeningococcus serum, but these hopes have not been realized. Amoss and Marsh,² and Neill and Taft³ found the method too irregular in its results and insufficient for a guide to the therapeutic potency and standardization of antimeningococcus serum.

Action of Antimeningococcus Serum.—As previously stated, experiments *in vitro* show that a potent antimeningococcal serum possesses three chief antibodies upon which its curative powers probably depend, namely: (1) *Bacteriotropins* (immune opsonins), which lower the resistance of the meningococci and facilitate their phagocytosis; (2) *bactericidins*, which kill the cocci extracellularly, either with or without final lysis; and (3) *antitoxins*, which neutralize the true extracellular toxin, which some strains of meningococci apparently produce in varying degree, and *anti-endotoxins*. Other than these are the *agglutinins*, which probably aid in bacteriolysis, and *anti-aggressins*, which may assist in the process of phagocytosis.

Microscopic examination of a direct stained smear of the sediment of cerebrospinal fluid obtained from fresh cases will show large numbers of polynuclear leukocytes and cocci, the majority of the latter being extracellular. As the case improves, whether under serum treatment or spontaneously, the micro-organisms diminish in number and become intracellular, frequently appearing clumped and failing to grow in culture. It would appear, therefore, that a cure is brought about partly by means of phagocytosis aided by bacteriotropins; by bacteriolysis through the agency of

¹ Jour. Immunology, 1916, 1, 345.

² Jour. Exper. Med., 1918, 28, 779.

³ Hygienic Laboratory Bulletin, 1920, No. 124, 93.

specific bacteriolytic amboceptors in the immune serum and complements in the spinal fluid and blood-serum, and to some extent by neutralization of a toxin with antitoxin.

A potent antimeningococcus serum furnishes these main antibodies, and since the first two must act locally upon the cocci infecting the meninges, the serum must be applied locally and directly by intraspinal and subdural injection, since only traces of immune serum could eventually find their way into the cerebrospinal fluid if the serum were injected subcutaneously or intravenously. On the other hand, in the treatment of meningococcus bacteremia and toxemia the serum should be injected intravenously and subcutaneously.

Polyvalent and Monovalent Sera.—Unfortunately, an immune serum may not contain the antibodies for the cocci producing a given infection, and hence the serum, even though it is skilfully administered in large doses, will have no influence upon the disease. Apparently the cocci of these resistant or “fast” strains are uninjured by the antibodies in the serum. To overcome this difficulty, a large number of different strains of meningococci are used in immunizing horses. In the Rockefeller Institute horses have been immunized with as many as 40 strains and during the recent war this serum yielded excellent results. If, however, the serum of one laboratory is found to exert no beneficial effect, the physician should use the serum of another, for different laboratories probably immunize their horses with cultures not in common use. *It is highly desirable to secure cultures of these “fast” strains. These should be sent at once to laboratories engaged in the production of antimeningitic serum, for the larger the number of these strains used in immunization, the more potent and valuable will be the serum.* Very probably the use of monovalent sera will still further improve the results of the serum treatment of this disease. Under these conditions the type of infection would first require determination by the agglutination test applied to cocci removed from the spinal fluid, the technic being similar to the agglutination test for the typing of pneumococci. Gordon and Hine¹ have recently published the results of the treatment of 90 cases by monovalent serum. The plan was to first inject a mixture of equal parts of Type I and Type II (Gordon’s classification) sera in order to lose no time while the typing was being done. Treatment was then continued with monovalent serum. Seven cases were fatal from other causes and may be excluded. The results with the remaining 83 were as follows:

- (a) 34, or 41 per cent., were due to Type I, with 1 death, or 3 per cent.
- (b) 32, or 38 per cent., were due to Type II, with 7 deaths, or 21.9 per cent.
- (c) 10, or 12 per cent., were due to Type III, with no deaths.
- (d) 7, or 9 per cent., were due to unknown types and 2 deaths, or 28.6 per cent.

The general mortality, therefore, was 12 per cent., which is an improvement on the general results of serum treatment of this disease. Type II serum was the least successful of these monovalent sera. This Type II group is more complex than the other groups and probably contains subgroups. Favorable reports on the use of monovalent sera have also been made by Banks² and Munro.³

The Importance of Early Diagnosis and Treatment.—Statistics have shown that the best results in the serum treatment of meningitis are secured

¹ Report of Medical Research Committee, January 28, 1919.

² Lancet, 1920, 1.

³ Brit. Med. Jour., March 27, 1920, 430.

in the early cases. Lumbar puncture and examination of the spinal fluid should always be done at once and without delay when symptoms are present suggestive of meningitis; this is especially true in times of epidemics.

When meningitis develops acutely and spontaneously without previous infection of the ear, mastoid or accessory sinuses, the infection is usually meningococcic, and I believe it is good practice to give the first dose of serum at the time of the first spinal puncture if the fluid is opalescent or cloudy. Bacteriologic diagnosis can usually be made within an hour by examination of smears of sediment of spinal fluid stained according to the method of Gram. Not infrequently, however, prolonged search may reveal only a few or none of the Gram-negative diplococci, although many pus-cells are present. These are usually meningococcus infections because in pneumococcus and streptococcus meningitis very large numbers of Gram-positive cocci are to be found. Cultures in the early cases are apt to prove sterile and if the smears are likewise negative or doubtful, twenty-four or more hours may be lost in treatment unless serum has been introduced with the first puncture. Even though the case does prove to be a pneumococcus or other infection, no particular harm has been done by the injection of the antimeningococcic serum.

It is true that experiments on the lower animals have indicated that when meningococci are in the blood their localization in the meninges may be favored by spinal puncture. This would tend to make the physician hesitate to do lumbar puncture. The writer believes, however, on the basis of experiments, that this danger is overemphasized unless the meninges are irritated by an injection of normal serum. When symptoms of meningitis are present I am sure spinal puncture should be done at once and a *good antiserum* injected.

Intraspinal Administration and Dosage of Antimeningitic Serum.—*In Acute Meningitis.*—As a rule, serum should be injected into the spinal canal as early in the disease as possible, and in such maximum amount as is compatible with safety. Intraspinal injection is absolutely necessary, for the serum must be brought into contact with the infected membranes, and only a trace would reach the spinal fluid if the serum were injected subcutaneously or intravenously. The advantages of early administration are obvious, and if the symptoms are indefinite, the physician should not hesitate to perform lumbar puncture and to secure fluid for microscopic examination, just as he would take a throat or nose culture to aid in the diagnosis of diphtheria. The maximum, or at least an adequate, amount of serum should be injected, care being observed to avoid undue pressure as a result of injecting too quickly or too large an amount. This administration of antimeningitic serum is, therefore, an important and delicate, though relatively simple, procedure.

1. The technic of intraspinal injection has been described on p. 850. Whenever possible, the serum should be injected by the gravity method, and the amounts of fluid withdrawn and serum injected controlled by blood-pressure readings.

2. Lumbar puncture is performed, and the fluid collected in graduated tubes. In the ordinary case fluid may be allowed to drain until the blood-pressure drops about 10 mm. of mercury, or if the pressure is unchanged or rises, until the fluid flows about 1 drop every three seconds, provided there are no other evidences of collapse, such as faintness, headache, and great restlessness.

3. As a rule, the amount of serum injected should be slightly less than the amount of fluid withdrawn. When the injection is controlled by the

blood-pressure readings—the amount varies considerably—usually the injection should stop when the pressure falls another 10 or 15 mm. For adults, the dose of serum should be about 30 c.c.; for an infant, about 15 c.c.

The serum should be allowed to flow in slowly, an ordinary injection consuming at least ten or fifteen minutes. If symptoms of collapse should appear before an adequate amount of serum has been injected, the funnel may be lowered and the spinal fluids allowed to flow out. When the symptoms have disappeared, the injections may be continued and satisfactorily completed.

3. When the physician cannot administer the serum by the gravity method or under blood-pressure control, the injection may be given by means of a syringe (see p. 855). It should be given slowly, and the patient observed closely in order to detect the general symptoms of collapse. The amount of serum injected should not be larger than the amount of cerebrospinal fluid withdrawn. According to Sophian, the average doses are as follows:

	DOSE OF ANTIMENINGITIC SERUM.	AMOUNT OF FLUID WITHDRAWN.
One to five years.....	3 to 12 c.c.	12 c.c.
Five to ten years.....	5 to 15 c.c.	15 c.c.
Ten to fifteen years.....	10 to 20 c.c.	20 c.c.
Fifteen to twenty years.....	15 to 25 c.c.	30 c.c.
Twenty years and over.....	20 to 30 c.c.	40 c.c.

The injection of too large a dose of serum may be followed by headache, pain in the back and legs, and restlessness. When the amount of serum injected exceeds the amount of spinal fluid withdrawn the symptoms just named must be regarded as the signal to stop; otherwise they may be disregarded.

Reactivation of Serum.—According to the experiments of Matsunami, Toyama, and the writer,¹ the addition of fresh normal human or guinea-pig serum to commercial antimeningitis serum increases its opsonic and bactericidal activities; Hektoen and Tunnickliff² have confirmed these observations in so far as opsonic activity is concerned. Since the spinal fluid is generally free of opsonins and complements, the writer has advocated³ the addition of fresh normal serum to antimeningitis serum before intraspinal injection. Independently Fairley and Stewart⁴ have made the same recommendations.

Unfortunately the trouble and labor involved retard the adoption of this form of treatment, but I believe it is of distinct value in the treatment of some cases that fail to improve and especially show in smears that phagocytosis of meningococci is not proceeding as rapidly as necessary. My procedure now is to remove 10 to 20 c.c. of blood from healthy older children or adults into a sterile centrifuge tube, secure the serum, and keep it on ice. When necessary 2 to 5 c.c. of this serum are added to the immune serum just before intraspinal injection.

Intravenous Administration of Antimeningococcus Serum.—During epidemics of meningitis it may be possible to detect cases in the bacteremic stage when meningococci are present in the blood and clear fluid is collecting in the ventricles. In these and in all severe fulminant infections it is good practice to inject from 30 to 100 c.c. of serum intramuscularly or intravenously. It is advisable to secure a culture of blood in ascites dextrose

¹ Jour. Immunology, 1918, 3, 157, 177.

² Jour. Infect. Dis., 1921, 29, 553.

³ Jour. Amer. Med. Assoc., 1918, 71, 1856.

⁴ Commonwealth of Australia, Service Publication No. 9.

broth in all cases in adults and older children. If sufficient serum may be obtained and the expense is a secondary consideration, an intravenous or intramuscular injection, given at the outset and once or twice during the acute stage, may benefit the patient by neutralizing toxins and possibly prevent complications due to the entrance of meningococci into the bloodstream. Furthermore, as shown by Flexner and Amos,¹ in poliomyelitis the intraspinal injection of serum increases the permeability of the choroid plexus and meninges, permitting the passage of immunity principles from the blood into the cerebrospinal fluid. For this reason the intravenous or intramuscular injection of antimeningitis serum in conjunction with intraspinal injections may be particularly advantageous in the treatment of severe infections.

With intravenous injections of serum care must be exercised against severe reactions. It is good practice to first inject subcutaneously 1 c.c. of the serum and give the balance about an hour later, injecting slowly. Blackfan² has directed particular attention to these reactions and advises against intravenous injections of serum for children.

Herrick³ has reported particularly good results from treatment by intravenous injections of serum in severe adult cases, the patients often coming out of coma with rapid recession of the rash and symptoms. The mortality was reduced from 64 to 19 per cent. Out of Herrick's 265 cases, 64, or 25 per cent., proved fatal, but out of 137 cases given ordinary intraspinal injections and less than 45 c.c. of serum intravenously 47, or 34 per cent., proved fatal, whereas out of 128 cases treated intraspinally with small doses and intravenously with large doses of serum 19, or 15 per cent., proved fatal.

Of the two forms of serum therapy—intraspinal and intravenous—the former is the more important. Acute primary meningococcus bacteremia without involvement of the meninges is rare, but when meningococci are found in the blood, serum should be given intravenously. In severe adult cases seen within the first three days serum may be given intravenously as a routine; at least one dose of 100 c.c. may be given.

Repeating Doses of Serum.—It is the general rule to give an intraspinal injection of serum every day for four days, and then on alternate days until the acute symptoms have subsided, and to resume the treatment if an exacerbation or a relapse occurs. In severe fulminant infections, and especially if the exudate is thick and only small amounts of serum can be introduced under pressure, it is well to repeat the injection every twelve hours until several doses have been given. Some cases require daily consecutive injections for six or more days; the average case will require from four to six injections if the treatment is begun during the acute stage; in the subacute and chronic cases many more treatments are required. There are two main indications and guides:

1. The condition of the cerebrospinal fluid.
2. The clinical condition of the patient.

1. In most instances the cerebrospinal fluid tends to clear up macroscopically as the disease improves. This is, however, occasionally misleading, as the fluid may become more turbid as the result of an *aseptic meningitis* or excitation of a polynuclear leukocytosis due to the serum, while, in reality, the numbers of meningococci are diminishing and the patient is improving.

¹ Jour. Exper. Med., 1917, xxv, 499, 525.

² Jour. Amer. Med. Assoc., 1921, 76, 36.

³ Jour. Amer. Med. Assoc., 1918, 71, 612.

More accurate information is obtained by the microscopic examination of a stained smear of the sediment of the cerebrospinal fluid withdrawn. In fresh acute cases the cocci are numerous and mostly extracellular; improvement is indicated by a diminution in their number, and by the fact that they are mostly intracellular. I generally determine the *phagocytic index* or the relative proportion of leukocytes that have engulfed cocci and the *opsonic index* or the relative number of cocci per leukocyte as determined by counting a large number. When many cocci are present, or if they are few in number but mostly extracellular, the indications are to puncture next day, even if the clinical condition of the patient is good and the temperature is lower. The number and position of cocci are, therefore, of more importance as a guide to subsequent injections than is the total number of pus-cells.

2. As an indication for repeating the doses of serum the clinical condition is of most value when combined with the examination of the cerebrospinal fluid. Occasionally the patient's condition may seem to improve, although the fluid may show numerous cocci, which will subsequently aggravate the clinical condition unless the serum is administered. In favorable cases there is usually a lower temperature the day following an injection, and frequently delirium becomes less marked and there is some return to consciousness. The complexion, which is often cyanosed at first, regains a healthy color; the pain in the head, neck, and limbs becomes less severe, although the neck and spine may remain stiff for several days. Finally the mind becomes clear and the patient is cheerful, and no longer irritable, apathetic, and hypersensitive. He feels better and his appetite returns. When this favorable outcome supervenes, the serum injections may be discontinued, to be resumed, however, upon the first evidence of a relapse. The physician should be on his guard for the appearance of acute hydrocephalus, which condition is relieved by repeated lumbar puncture.

The Serum Treatment of Cases with Thick Plastic Exudate.—In very severe cases the exudate may be so thick that it will not flow from the needle. In these cases the serum should be injected in small doses under pressure, and the injections repeated every eight to twelve hours. As they are likely in any case to terminate fatally, the physician is justified in taking the risk of increasing intracranial pressure. It may be well carefully to inject a small amount of warm sterile salt solution, which will dilute the exudate and possibly start a flow; or a second needle may be inserted higher up, when a thinner exudate is found, or washing may be possible by injecting salt solution in the upper needle and draining through the lower.

The Serum Treatment of Cases with a Dry Canal and Cases of Posterior Basal Meningitis.—Occasionally a patient improves clinically and the amount of cerebrospinal fluid becomes very scanty, the spinal tap being dry, although it is certain that the needle has entered the subarchnoid space. In such instances a small amount of serum may be injected, or the injection may be dispensed with if the clinical condition continues to improve. If, however, cases with dry canals present evidences of toxemia and general aggravation of symptoms, small doses of serum should be injected under pressure and the injections repeated as often as necessary. The physician must be very cautious, however, for if there are clinical evidences of severe intracranial pressure, it is probable that there is an encapsulation of fluid within the ventricles, and shutting off of the communication between the ventricles and the subarchnoid space. In this *posterior basic meningitis* intraspinal injections are dangerous and aggravate the process. In infants it is necessary to puncture the ventricles through the anterior fontanel as

first practised by Cushing and Sladen,¹ and in older children and adults by trephining at Kocher's point, removing the fluid, and if it is found to be cloudy or purulent, injecting serum. It may be necessary to tap both ventricles alternately at intervals of several days, depending upon the reaccumulation of fluid and pressure symptoms. Permanent drainage may be instituted in severe cases by means of small catheters. While the operation is not usually dangerous, the ultimate prognosis is very unfavorable.

In adults trephining and injecting serum into the ventricles has yielded, according to Marcland,² 4 cures among 18 cases. Landry and Hamley³ had 1 cure out of 5 cases, but Fairley and Stewart lost all of 9 cases. In place of trephining and injecting the serum Herrick recommends Cobb's method of breaking down adhesions around the foramen magnum and fourth ventricle by manipulations of the head under general anesthesia to relax the neck muscles. This method may produce an increased flow of spinal fluid.

Cistern puncture and injection of serum may also be tried. Ayer⁴ has conducted the puncture in 20 cases without mishap and his method is described in Chapter XXXVII. Recently Mitchell and Reilly⁵ have recorded the recovery of an infant of four months following repeated cistern punctures and drainage and the injection of 5 to 8 c.c. of serum each time.

The Serum Treatment of Subacute and Chronic Meningitis.—If there is no evidence of sepsis; if the mind is clear and the neck limber; if the general conditions are good and the cerebrospinal fluid is practically cleared up, the affection is most likely hydrocephalic, and may be relieved by repeated spinal punctures, with removal of as much fluid as is safe, using blood-pressure as an index. If meningococci are present in cultures of the fluid, small amounts of serum may be injected. The prognosis in these cases, however, is generally bad, as the process is prolonged and the patient finally succumbs.

In the second form of chronic meningitis, when the meningeal symptoms are active, intensified, and persistent, serum should be administered every few days in the same manner and in the same dosage as in the acute cases. Improvement is, however, usually temporary, and the ultimate prognosis is very grave.

Serum Sickness.—Intraspinal injections of serum result in the sensitization of the patient in just the same manner as if serum were injected by other routes. The percentage of cases developing serum sickness is likely to be high, since antimeningitic serum is not refined (Sophian reports 60 per cent. of his cases as developing the condition), and while the symptoms are distressing, they are seldom alarming, and fatal anaphylaxis is extremely rare. Occasionally the onset of serum sickness may be mistaken for a recurrence of meningitis, but if the meningeal condition has been responding as well as could be expected, it is wise to let the patient alone, rather than to make additional punctures and cause further depression. Local sedatives, laxatives, atropin, sedatives, and, at times, morphin, are indicated (see Chapter XXXII).

Summary and General Plan for the Serum Treatment of Meningococcus Meningitis.—For cases of average severity first diagnosed on or about the third day of the disease the following plan of serum treatment is advised:

¹ Jour. Exper. Med., 1908, 10, 548.

² Bull. et mém. Soc. d. hôp., 1918, xlii, 1218.

³ Amer. Jour. Med. Sci., 1919, 157, 210.

⁴ Arch. Neurol. and Psych., 1920, 4, 529.

⁵ Amer. Jour. Med. Sci., 1922, 164, 66.

spinal fluid should always be drained off, in amounts slightly more than the amount of serum injected. If the spinal fluid pressure is high, it is well to drain spinal fluid until it flows at the rate of about 1 drop in five seconds. *Each spinal fluid should be examined for meningococci.*

First day: For children under twelve years: 15 c.c. of serum intraspinally at the time of first spinal puncture or as soon as possible thereafter. Repeat the drainage and injection of serum eight to twelve hours later.

For children over twelve years and adults: 20 to 30 c.c. of serum intraspinally as soon as possible. Blood culture. Severe cases in adults to receive 100 to 150 c.c. serum intravenously one hour after the subcutaneous injection of 1 c.c. of the serum for desensitization. Intraspinial injection to be repeated eight to twelve hours later.

Second day: Repeat the intraspinal injection. If very severe give a second intraspinal injection eight to twelve hours later. If blood culture is positive, give a second intravenous injection of serum.

Third and fourth days: One intraspinal injection of serum. If patient is not improving or growing worse, give a second injection on these days about eight to twelve hours later.

Fifth day: If spinal fluid removed in fourth day shows no meningococci, spinal puncture may be done and fluid removed if it is under pressure, but serum may be omitted. As a general rule, however, it is well to inject serum intraspinally.

The appearance of the fluid if cloudy may be misleading because leukocytes may be present by reason of irritation of the meninges by the serum and meningococci absent.

Serum should be injected intraspinally at least once a day until two successive spinal fluids have proved sterile. At that time serum may be stopped, but a sharp watch kept up for relapses. Serum sickness will probably develop and should not be mistaken for a relapse.

Results of the Serum Treatment on Meningococcus Meningitis.—

(a) *Upon the Course of the Disease.*—In the majority of cases the subdural injection of a potent antimeningitic serum is followed by some immediate improvement in the local suppurative meningitis and general sepsis, for the temperature usually drops, the mental condition improves, and delirium is diminished, although the Kernig sign may persist, partly as the result of meningeal irritation and partly on account of fear. *Hydrocephalus* is generally relieved, as indicated by lessening of the pressure symptoms, as, for example, severe headache, vertigo, and vomiting; breathing becomes more regular, and the pulse also becomes slower and more regular. The duration of the illness is usually shortened. According to Holt, in the New York epidemic of 1904-05, antedating the use of serum, among 350 cases that recovered the duration in 3 per cent. was one week or less, and in 50 per cent. five weeks or longer. Of 288 cases reported by Flexner and Jobling, the average duration of active symptoms in those receiving serum was eleven days. Sophian, in an experience of several hundred cases, reports that many acute cases were relieved in five or six days and discharged as cured in two weeks.

(b) *Upon Complications.*—Next to its influence upon mortality, the good effects of antimeningitic serum are apparent in that it lessens the incidence and severity of the terrible complications of this disease. The most severe and permanent sequels are those resulting from affections of the internal ear and the essential structures of vision. A conservative estimate of the incidence of the former among cases not receiving serum treatment

is 12 per cent. (Göffert), whereas Flexner's¹ analysis of 1300 cases treated with serum shows that deafness occurred in but 3.5 per cent. of cases. At least from 12 to 24 per cent. of cases not receiving serum treatment will develop more or less serious eye complications, whereas Flexner's report shows that impairment of vision among serum-treated cases occurred in about 0.9 per cent. of cases. The latter report shows the occurrence of arthritis in but 0.9 per cent. of cases, whereas among cases untreated with serum this is a frequent complication. Whereas chronic meningitis is relatively common among cases treated without serum, it is uncommon among those treated with serum.

(c) *Upon Mortality.*—The gross mortality among cases treated without serum varies from 70 to 90 per cent.; among serum-treated cases the mortality is about 30 per cent. For example, of 1294 cases treated with serum prepared in the Rockefeller Institute, the general mortality was 30.9 per cent.

The importance of early diagnosis and prompt institution of serum treatment is shown in the following table:

MORTALITY ACCORDING TO THE PERIOD OF INJECTION OF SERUM

TIME OF INJECTION.	MORTALITY, PER CENT.			
	Flexner, 1294 Cases.	Dopter, 402 Cases.	Netter and Debre, 99 Cases.	Sophian, 161 Cases.
First to third day.....	18.1	8.2	20.9	9.0
Fourth to seventh day.....	27.2	14.4	33.3	14.9
Later than seventh day.....	36.5	24.1	26.0	22.6
Average mortality.....	30.8	16.44	28.0	15.5

The influence of age upon mortality was early pointed out by Flexner. The very high mortality in infants and in old persons is due to their lowered vitality and enfeebled resistance. An additional factor in young children is their greater tendency to develop extreme hydrocephalus and convulsions.

MORTALITY ACCORDING TO AGE

AGE.	REPORTED BY			
	Flexner.	Dopter.	Netter.	Sophian.
Under one year.....	49.6	48.6	50.0	50.0
One to two years.....	31.0	20.1	0.0	21.2
Two to five years.....	28.4	9.3	16.6	17.5
Five to ten years.....	15.1	8.5	12.5	9.0
Ten to twenty years.....	29.4	10.2	0.0	18.0
Over twenty years or age not given.	38.2	14.1	0.0	32.0

The statistics show indubitably that the mortality of epidemic meningitis can be greatly reduced by the administration of serum. While the ordinary type of epidemic meningitis responds best to the specific treatment, the fulminant cases may also receive some of the beneficial influence of the serum. To quote from what Flexner wrote in 1909, and repeated in

¹ Jour. Exper. Med., 1913, xvii, 553.

1913: "In view of the various considerations presented, the conclusions may be drawn that the antimeningitis serum, when used by the subdural method of injection, in suitable doses and at proper intervals, is capable of reducing the period of illness; of preventing in large measure the chronic lesions and types of the infection, of bringing about complete restoration of health, thus lessening the serious, deforming, and permanent consequences of meningitis; and of greatly diminishing the fatalities due to the disease."

During the great war meningococcus meningitis was of wide-spread occurrence. During the first year or two a sudden demand for serum was met by supplies of poor quality sera, but this situation was soon corrected with an improvement in results and a lowering of the mortality to the neighborhood of 20 per cent.

Treatment of Meningitis with Human Serum.—MacKenzie and Martin¹ in 1908 treated 16 acute cases with intraspinal injections of 15 to 20 c.c. of fresh sterile human serum, with a mortality of 38 per cent.

Cases of meningitis have also been treated with intraspinal injections of serum from convalescent cases of the disease as well as by injections of the patient's own serum.

Convalescent serum may doubtless prove worth administering in exceptional cases, if derived from an individual recovered from the same type of infection, but the curative value of normal human serum is questionable, although its addition to antimeningococcus serum may prove distinctly worth while in the treatment of some cases, as previously discussed, by increasing the opsonic and bactericidal activity of the latter.

Vaccine Treatment of Meningococcus Meningitis.—Vaccines have been advocated in the treatment of subacute and chronic cases when serum appears to be losing its effect. Rolleston² reports the treatment of 21 cases with vaccines, always in addition to other treatment, with a mortality of 23.5 per cent.

Crowe³ considered autogenous vaccines of aid in treatment and began early in the acute stage with small doses of 1,000,000 cocci. Hall,⁴ Collins,⁵ Sophian,⁶ Colebrook,⁷ Fairley and Stewart,⁸ Worster-Drought and Kennedy,⁹ Chalmers and O'Farrel,¹⁰ MacLagan,¹¹ and others have also reported favorably upon the use of vaccines in subacute and chronic cases of the disease.

The vaccines should be autogenous; if a stock vaccine is used it should be polyvalent in order to make sure that it includes the type producing the disease. In subacute and chronic cases the vaccine may contain 1,000,000,000 cocci per cubic centimeter. Subcutaneous injections may be given every five days beginning with 0.1 c.c. for persons fifteen years or older and gradually increasing until 1 c.c. is being given at one time.

THE SERUM TREATMENT OF INFLUENZAL MENINGITIS

Since lumbar puncture as an aid to the diagnosis of meningitis is coming into more general use, the important fact has been revealed that the influenza

¹ Jour. Path. and Bacteriol., 1908, 12, 539.

² Lancet, 1919, 1, 645.

³ Lancet, 1915, 2, 1127.

⁴ Medical Research Committee Report, 1916.

⁵ Brit. Med. Jour., 1915, 1, 287.

⁶ Epidemic Cerebrospinal Meningitis, C. V. Mosby Co., 1913, 192.

⁷ Lancet, 1915, 1, 1026.

⁸ Commonwealth of Australia Service Publication, No. 9, 1916.

⁹ Cerebrospinal Fever, A. and C. Black, London, 1919, 428.

¹⁰ Jour. Trop. Med. and Hyg., 1915-16, xl, 101.

¹¹ Edinb. Med. Jour., 1918, 20, 375.

bacillus is not an infrequent cause of severe, and usually fatal, seropurulent cerebrospinal meningitis. In 1911 Wollstein¹ collected 58 cases of this infection, all but 6 ending fatally, and as the bacterial diagnosis of meningitis is becoming more widely known and more commonly employed, the number of reported cases is increasing rapidly. The mortality of 90 per cent., which is exceeded only by the tuberculous and pneumococcus infections of the meninges, and the encouraging results following the use of a specific anti-influenzal serum in experimental infections in monkeys, render this subject one of great importance from the standpoint of serum therapy.

Influenzal Meningitis.—Like the acute meningeal infections in general, influenzal meningitis is more prevalent among children than among adults.

Infection of the meninges is probably always secondary to infection of the respiratory tract with virulent influenza bacilli, the route of infection being chiefly through the blood-current. Direct infection from the nose cannot be excluded, and should be considered a possibility. However, all or nearly all cases of spontaneous influenzal meningitis in human beings are the result of influenzal bacteremia, since the bacilli have been cultivated in large numbers from the heart's blood before and after death. The same is true of experimental influenzal meningitis in the monkey.

According to Flexner,² the *cerebrospinal fluid* removed by lumbar puncture from human patients is always turbid, and deposits a yellowish or whitish sediment on standing. "As the disease advances, the fluid becomes more heavily charged with pus-cells, until toward the end, and as late as the seventh day of illness, the puncture may yield merely a viscid mass of purulent matter. The number of influenza bacilli present in the fluid is usually large, and the bacilli lie chiefly extracellular among the pus-cells, although a variable but small number is usually found ingested by the leukocytes. In morphology the bacilli vary somewhat, and in this respect the observer may readily be deceived as to the nature of the bacteria present. While some of the fluids contain the typical, minute rods, others show quite irregular and knobbed or even filamentous bacteria that have little resemblance to the influenza bacillus as seen in recent cultivations. These bizarre or *involution* forms, however, are met in old and exhausted cultures; and when they are recultivated on a suitable hemoglobin medium, they yield the typical minute rods."

The cerebrospinal fluid removed from monkeys inoculated by subdural injection with virulent cultures of the influenzal bacillus resembles in all essential particulars the fluid removed from patients with spontaneous infections.

The *bacteriologic diagnosis* can usually be made by microscopic examination of stained smears of the fluid, but whenever possible, the diagnosis should be confirmed by cultural methods.

Anti-influenza Serum.—After having satisfactorily demonstrated experimental influenza meningitis in the monkey, Flexner and Wollstein prepared an immune serum and showed that the experimental infection could be controlled and cured by injecting the serum directly into the seat of disease by intraspinal inoculation. The immune serum was prepared by the ordinary methods, first a goat and then a horse being injected with non-virulent and finally with virulent bacilli, covering a period of many months, until their serums showed the presence of agglutinins and bacteriotropins. The serum lacked bacteriolytic properties, and did not give rise to complement fixation in dilutions greater than 1 : 100.

¹ Jour. Exper. Med., 1911, xiv, 73; Amer. Jour. Dis. Child., 1911, i, 42.

² Jour. Amer. Med. Assoc., 1913, lxi, 1872.

Following the administration of serum, the cerebrospinal fluid tends to clear up; the bacilli become fewer in number and are mostly ingested by the phagocytes; the pus-cells become less numerous, and while the bacilli may persist in the fluid for a longer period, they ultimately disappear.

Administration of Anti-influenza Serum.—The Rockefeller Institute has distributed serum throughout different parts of the country, and is prepared to furnish it to physicians upon request. *Physicians, and especially pediatricists, should resort to lumbar puncture early in all suspected cases of meningitis, for only in this manner may influenzal meningitis be detected early to derive any possible benefit from serum treatment. The serum should be injected directly into the spinal canal by the gravity or syringe method in exactly the same manner and with the same precautions as are observed in administering serum in the treatment of epidemic meningitis.* Since the disease is usually accompanied by a bacteremia, it is well to inject serum *intravenously*, although serum injected *intraspinally* soon finds its way into the blood-stream.

TREATMENT OF PNEUMOCOCCUS MENINGITIS

Meningitis is caused more frequently by the pneumococcus than by the influenza bacillus. Its mortality is certainly no less than in influenzal meningitis. During the past four years I have seen 24 cases and all were fatal.

The few instances in which antipneumococci serum has been employed have not yielded results that inspire confidence in its employment alone. Even when the homologous serum is used in treating experimental pneumococcus meningitis in monkeys, the fatal termination may be delayed, but is not prevented. For this reason the outlook for its successful employment alone in human infections is not encouraging. Investigations by Lamar¹ have shown, however, that mixtures of homologous antipneumococcus serum, sodium oleate, and boric acid exert a marked and decided curative influence upon a virulent experimental meningitis, and while this method has not thus far been generally applied in the treatment of the disease in humans, it is deserving of trial and offers some encouragement for an otherwise highly fatal infection.

More recently Litchfield² and Gray³ have reported the successful treatment of the disease with Kyes' fowl antipneumococcus serum, to which further reference will be made.

The Nature of Pneumococcus Meningitis.—This infection is usually secondary, and follows on pneumonia or on inflammations of serous membranes by indirect transmission by the blood or by direct infection from the nasopharynx, mastoid cells, frontal, sphenoid and ethmoid sinuses, and internal ear.

The *diagnosis* is usually made as the result of microscopic examination of stained smears of cerebrospinal fluid removed by lumbar puncture. Large numbers of polynuclear leukocytes with intracellular and extracellular Gram-positive diplococci, occurring in pairs or in short chains, usually indicate a pneumococcus infection. Whenever possible, the diagnosis should be confirmed by making cultures of the fluid on dextrose blood-agar, and by injecting portions intraperitoneally and subcutaneously in mice.

Pneumococcus infections of the cerebral meninges have been found

¹ Jour. Exper. Med., 1911, i; *ibid.*, 380; xiv, 256; 1912, xvi, 581.

² Jour. Amer. Med. Assoc., 1919, 72, 1345.

³ Amer. Jour. Med. Sci., 1920, 159, 885.

experimentally to be more refractory to treatment than infections of the spinal meninges, hence human infections following injuries to the head, or occurring as the result of direct extension from neighboring sinuses, are likely to be more refractory than indirect infections by way of the blood.

Importance of Early Diagnosis and Drainage.—*Early diagnosis by spinal puncture and examination of spinal fluid is of great importance; ordinarily much valuable time is lost.* The exudate in pneumococcus meningitis rapidly thickens and greatly hinders drainage by spinal puncture. The spinal fluid contains untold millions of pneumococci which proliferate rapidly and soon overwhelm the patient. The writer regards pneumococcus meningitis among the most fatal of infections, and in view of its frequency and particularly after infections and operations upon the mastoid cells and accessory nasal sinuses the disease is one urgently demanding investigation for the purpose of evolving an efficient treatment.

Some means should be found for affording drainage. Something in this direction can be accomplished by two or more spinal punctures daily, removing as much spinal fluid as possible. Likewise washing out the spinal canal between a needle in the upper dorsal and one in the lower lumbar regions, may aid. But the blunt of the attack is in the cerebral subarachnoid space and sera introduced in the lumbar region are soon blocked from diffusion to these parts by the plastic exudate. Cistern puncture (described in Chapter XXXII) and drainage is to be thought of in this connection; also washing out of the meningeal cavities by puncture of the lateral ventricles, as described by Bellin and his associates.¹ Some means should be devised for more or less continuous drainage of the cerebral and spinal meninges with facilities for flushing out the subarachnoid space at intervals with saline solution, serum, or some chemical agent. The successful institution of continuous drainage will open up hope for success in the treatment of this dreadful disease and the writer and his associates are now investigating the problem.

Treatment with Antipneumococcus Serum, Sodium Oleate, and Boric Acid.—Numerous investigations by Conradi,² Korschun and Morgenroth,³ Levaditi,⁴ and Noguchi⁵ have shown that substances may be obtained directly from tissue-cells and leukocytes or after autolysis which are bactericidal and hemolytic, and, as shown by Noguchi, are largely in the nature of higher saturated fatty acids or their alkaline soaps. As shown by Klotz,⁶ soaps occur in inflammatory foci, and the origin of the fatty acids and soaps is readily accounted for since Achaline⁷ has shown the presence of lipase in such foci. With the death of leukocytes in an inflammatory focus, brought about by a bacterial poison, leukocidins, or lack of nutriment, disintegration occurs, and by autolysis and lipolysis fatty acids and soaps are produced that in themselves seem to exert a destructive action upon the infecting bacteria.

With these considerations in mind, Lamar investigated the influence of soaps upon pneumococci. Solutions of 0.5 to 1 per cent. of sodium oleate were found rapidly to kill pneumococci; much weaker solutions, as, e. g., 0.1 per cent., or even 1 part of soap in 10,000 parts of water, were found to lessen their virulence, and, what is more important and significant, to

¹ Lyon Chirurgical, 1918, 15, 455.

² Beitr. z. chem. Phys. u. Path., 1902, i, 193.

³ Berl. klin. Wchn., 1902, xxxix, 870.

⁴ Ann. de l'Inst. Pasteur, 1903, xvii, 187.

⁵ Biochem. Ztschr., 1907, vi, 327.

⁶ Jour. Exper. Med., 1905, vii, 633.

⁷ Compt. rend. Soc. de biol., 1899, li, 568.

render the organisms peculiarly susceptible to lysis by a homologous antipneumococcus serum.

A serious drawback to the application of these discoveries was that protein constituents of serum and exudates were found to inhibit the bacteriolytic and hemolytic action of unsaturated fatty acid soaps, as was shown by Noguchi¹ and then by von Liebermann.² The latter and von Fenyvessy³ later found that this inhibition can be prevented in the test-tube and also in the animal body by adding a minute quantity of *boric acid*, which prevents the union of soap and protein matter when the latter is not too greatly in excess.

The experiments of Lamar with mixtures of homologous antipneumococcus serum, sodium oleate, and boric acid in the treatment of pneumococcus meningitis in the monkey have yielded excellent results, especially when used early in the infection. These experiments have proved that sodium oleate lowers the virulence of pneumococci and renders them peculiarly and highly susceptible to solution by bacteriolytins present in the serum, and that boric acid largely prevents the inhibitory action of protein constituents upon this sensitizing action of sodium oleate.

One drawback to the use of this method in the treatment of human infections is the necessity of using an antipneumococcus serum corresponding to the organism causing the infection. But a polyvalent serum may be employed or the type of infection quickly determined by agglutination reactions.

It is highly desirable that the treatment be administered as early as possible, when the exudate is largely serous or at most seropurulent. The mixture should be injected into the spinal canal after the withdrawal of the fluid by the gravity or syringe method and under blood-pressure control, as previously described. The amount injected and the number of injections depend upon the clinical condition of the patient, and in general may be administered in the same way as is antimeningitic serum.

The initial dose may be 20 c.c., and is prepared as follows:

Antipneumococcus serum (sterile)	4 c.c.
5 per cent. aqueous solution of boric acid (sterile)	15 c.c.
2 per cent. aqueous solution of sodium oleate (Kahlbaum's or Merck's) (sterile)	1 c.c.

Ordinarily two spinal punctures should be made daily and as much spinal fluid removed as possible; the mixture or antipneumococcus serum alone should be injected after each drainage.

Intravenous injections of serum are also advisable not only in the treatment of meningitis developing during the course of pneumonia, but also in that more fatal form developing by direct extension from the mastoid cells and accessory sinuses. For this purpose 50 to 100 c.c. may be administered as described in the serum treatment of pneumonia.

Treatment with Kyes Chicken Antipneumococcus Serum.—During the epidemic of influenza in 1918 Litchfield⁴ treated 10 cases of pneumococcus meningitis developing in the course of lobar pneumonia. Kyes' antipneumococcus serum was injected intraspinally and intravenously, with 5 recoveries. Gray,⁵ subsequently reported an additional case treated with this serum, with a fatal outcome.

¹ Biochem. Ztschr., 1907, vi, 327.

² Biochem. Ztschr., 1907, iv, 25.

³ Ztschr. f. Immunitätsf., orig., 1909, ii, 436.

⁴ Jour. Amer. Med. Assoc., 1919, 72, 1345.

⁵ Amer. Jour. Med. Sci., 1920, 159, 885.

Treatment with Mixtures of Antipneumococcus Serum and Ethylhydrocuprein Hydrochlorid.—Morgenroth and Levy¹ have shown that optochin (ethylhydrocuprein) hydrochlorid, a synthetic compound of quinin, is highly pneumococcidal. These observations have been amply confirmed by Moore,² Cohen, Heist, and the writer,³ and others. Idzumi and the writer⁴ have found that the intraspinal injection of this drug has a favorable influence upon experimentally produced pneumococcus meningitis in dogs and rabbits when given *early* (within first thirty-six hours).

The drug and further observations on its use in the treatment of pneumococcus infections will be discussed in my monograph on Chemotherapy, but it is mentioned here for the possible benefit it may have in the treatment of pneumococcus meningitis of human beings.

A 1 : 200 solution is prepared in sterile saline solution and heated at 60° C. for one hour. For children under fifteen years of age a mixture of 5 c.c. of this solution and 10 c.c. of polyvalent antipneumococcus serum may be injected intraspinally twice daily after thorough drainage of spinal fluid. For adults 10 c.c. of the solution and 20 c.c. of serum should be injected twice daily for three or four days. The internal administration of ethylhydrocuprein also raises the pneumococcidal power of the blood and for an adult 0.5 gm. may be given three times daily in capsules. To the above mixtures for spinal injection I sometimes add 2 to 5 c.c. of fresh chicken serum for the purpose of reactivating the immune serum and for the natural immune substances in fowl serum.

SERUM TREATMENT OF STREPTOCOCCUS MENINGITIS

Streptococcus meningitis is much less common than pneumococcus meningitis. The disease almost invariably develops by extension to the cerebral meninges from suppuration foci in the mastoid cells, middle ear, or accessory nasal sinuses. Personally I have never seen streptococcus meningitis develop spontaneously, that is, without direct extension from these neighboring parts.

Bacteriologically the infection is readily mistaken for pneumococcus meningitis.

Treatment, such as it is, may be along the same lines as described for pneumococcus meningitis. The prognosis is always extremely grave. Spinal puncture should be done once or twice daily to release pressure and remove pus and organisms. Antistreptococcus serum may be injected intraspinally twice a day in dose of 10 to 30 c.c. I believe something is to be gained by adding to each dose of antistreptococcus serum 2 to 5 c.c. of fresh sterile human serum, for the purpose of reactivating the immune serum.

TREATMENT OF TUBERCULOUS MENINGITIS

A great many drugs and methods have been advocated for the treatment of this highly fatal disease. Hollis and Pardee⁵ have recently renewed the literature and report that 38 authentic and 15 doubtful cases of tuberculous meningitis have been recorded as cured, including 2 cases of proved and 2 doubtful cases of their own treated with intraspinal injections of *anti-meningococcus serum*. They also report on 3 additional cases treated with this serum, but with a fatal outcome in all. These cases, in addition to

¹ Berl. klin. Wchn., 1911, 48, 1561, 1650, 1779, 1983.

² Jour. Exper. Med., 1915, 22, 551.

³ Jour. Infect. Dis., 1917, 20, 313.

⁴ Jour. Infect. Dis., 1920, 26, 355.

⁵ Arch. Int. Med., 1920, 26, 49.

the one reported by Schaeffer,¹ makes a total of 8 treated with intraspinal injections of antimeningococcus serum, 5 of whom recovered.

Treatment consists of spinal puncture, drainage of the fluid and the injection of 15 to 30 c.c. of antimeningococcus serum once a day for three or four days, and thereafter at intervals of two or three days for two or more injections. The beneficial effects are probably entirely non-specific and mainly due to the local irritation of the meninges with the outpouring of serous and cellular elements and principally polymorphonuclear leukocytes. Probably the same effects could be secured by the injection of sterile normal horse-serum.

Tilli² has reported the recovery of 1 case of tuberculous meningitis in a child ascribed to the subcutaneous injection every three days of 1 to 3 c.c. of its own spinal fluid (autoserum).

Manwaring³ has observed that the intraspinal injection of extracts of dog leukocytes had some slight beneficial influence upon the course of experimental tuberculous meningitis of dogs. The injection of extracts of horse leukocytes had the effect of slightly delaying the development of the disease. The injection of rabbit leukocytes into experimentally infected monkeys also had a tendency to prolong the infection. These results suggest that it may be worth while to inject *leukocytic extracts* (commercially prepared of horse leukocytes) in the treatment of this highly fatal disease; for a child the dose may be 5 to 10 c.c. by intraspinal injection.

TREATMENT OF ANTHRAX

Infection and Immunity in Anthrax.—Anthrax is a disease of the lower animals and especially of sheep and cattle, transmissible to man. Natural immunity among the lower animals varies greatly; thus mice, guinea-pigs, and rabbits are more susceptible than sheep and cattle, while the dog and rat are relatively immune and fowls and cold-blooded animals highly immune.

Infection of man and the lower animals usually takes place by the entrance of virulent spores in the hair follicles or injuries of the skin; the resulting lesion is known as the "malignant pustule." Spores may be inhaled in dust and produce lesions in the respiratory tract, designated as "wool-sorters' disease." Bacilli or spores may be swallowed, escape destruction by the gastric juice and produce intestinal lesions. The latter infections are highly fatal forms of anthrax. Man is usually infected by handling hides, hair, and wool contaminated with the spores, which may survive drying or immersion in pickling fluids and brines for long periods of time.

For susceptible animals the anthrax bacillus is highly invasive, that is, capable of surviving in the body fluids and rapidly multiplying in the lymphatic and vascular channels. Curiously the presence of exogenous and endogenous toxins have not been conclusively demonstrated in cultures, although the general or bacteremic infection is accompanied by symptoms and degenerative changes in the parenchymatous organs characteristic of acute toxemia and especially in the terminal stages of the disease, when the very rapidly developing toxemia is overwhelming.

Natural immunity to anthrax and recovery from infection are apparently largely due to phagocytosis, as claimed by Metchnikoff and supported by the experiments of his associates. At least the presence of bactericidal substances in the serum does not appear to bear a relation to immunity, inas-

¹ New York Med. Jour., January 11, 1913.

² Policlinico, 1916, 33, 1357.

³ Jour. Exper. Med., 1912, 15, 1; *ibid.*, 1913, 17, 1.

much as the serum of the susceptible rabbit may be bactericidal, and that, of the resistant dog and rat almost lacking in bactericidal activity. Opsonins in the serum have been shown by Wright to possess an important bearing upon immunity by reason of their relation to phagocytosis. Anthrax bacilli are also able to produce an aggressin or toxic substance exerting negative chemotaxis; a part of the action of antianthrax serum is the neutralization of these substances which thereby facilitates phagocytosis.

One attack of anthrax does not confer a lasting immunity against the infection.

Antianthrax Serum.—The production of antianthrax serum by the immunization of horses or other animals with the bacilli and spores was among the earliest attempts in the development of serum therapy; owing to the virulence of the spores, the high invasiveness of the bacilli for the blood-stream, and the susceptibility of the horse to infection the production of immune sera is a matter of some difficulty and especially since the horse produces antibodies for anthrax rather slowly and seldom to a high degree.

Marchoux¹ produced a sheep antiserum that protected rabbits against fatal doses of anthrax, but Sclavo² was first to produce the serum on a large scale for the treatment of human beings. Sclavo immunizes animals over a long period of time, sometimes as long as two years. This serum has been extensively used in Europe and South America and to some extent in this country.

In the United States antianthrax serum has been produced by Eichhorn, Berg and Kelser³ in the Bureau of Animal Industry. Horses are immunized with injections of living spore vaccine while protected against infection with injections of anthrax serum. Great care is exercised to prevent contamination of the serum and elaborate tests made to insure the absence of anthrax spores. Eichhorn and his associates⁴ have succeeded in concentrating the serum in the same manner as diphtheria antitoxin, finding that the immune principles are carried down in the globulin fractions.

Antianthrax serum is now being prepared by the different manufacturing firms and is readily available for the treatment of the disease.

The literature on the results of the serum treatment of anthrax is quite large and generally favorable to the curative value of the serum for anthrax in human beings and the lower animals. Regan⁵ has recently published a good summary.

According to Sclavo the general mortality of anthrax in Italy without serum treatment is about 24 per cent.; with serum treatment the mortality is lowered to about 6 per cent. Mendez reports a mortality of 4.19 per cent. among cases treated with his serum.⁶ The reports of Cicognani,⁷ Legge,⁸ Herley,⁹ Royer and Holmes,¹⁰ Schwartz,¹¹ Regan,¹² Regan and Regan,¹³ Symmers,¹⁴ Hubbard and Jacobson,¹⁵ and numerous others indicate that the

¹ Ann. de l'Inst. Pasteur, 1895, 9, 785.

² Berl. klin. Wchn., 1901, 18, 19, 481, 520.

³ Jour. Amer. Vet. Assoc., 1915-16, xlvii, 669.

⁴ Jour. Agricult. Research, 1917, 8, 37.

⁵ Amer. Jour. Med. Sci., 1921, 162, 406.

⁶ Centralbl. f. Bakteriöl., 1899, 26, Nos. 21 and 22.

⁷ Gaz. d. osped. e. d. chir., 1901, No. 114.

⁸ Brit. Med. Jour., March 18, 1905, 589. ⁹ Lancet, 1909, 2, 1662.

¹⁰ Penna. Med. Jour., 1907, 2, 937.

¹¹ New York Med. Jour., 1918, cvii, 1171.

¹² Jour. Amer. Med. Assoc., 1919, 72, 1724.

¹³ Amer. Jour. Med. Sci., 1919, 157, 782.

¹⁴ Weekly Bulletin of New York Health Depart., August 7, 1920.

¹⁵ Monthly Bulletin of New York Health Depart., November, 1920.

administration of potent antiserum is effective in reducing the general mortality of anthrax.

The serum may be injected locally around the base of the malignant pustule in anthrax of the skin as advocated by Regan and doubtless aids in the processes of phagocytosis, but local anthrax is not particularly dangerous and various other forms of treatment may be employed with success. From the standpoint of serum treatment most interest attaches to the treatment of anthrax bacteremia and internal types of the disease (pulmonary and intestinal) which are accompanied by a mortality of 90 to 100 per cent. Pied¹ in 1913 states that up to that date he found reports of 7 cases of anthrax bacteremia treated with serum, with recoveries in 5 cases. Bandi,² Bissel,³ Graham and Detweiler,⁴ and others have reported recoveries of blood infections under serum treatment.

Treatment of Anthrax of the Skin (Malignant Pustule).—This is the usual form of anthrax in human beings and generally occurs among workers in hair, wool, and hides. The disease has also been caused by infected shaving brushes, hair brushes, and other articles made of hair.

Doubtless many cases of skin anthrax escape diagnosis and are treated by simple incision, drainage, and the application of wet dressings as in the surgical treatment of any local pyogenic infection.

However, the marked edema and involvement of the neighboring glands are apt to lead to bacteriologic examinations by smear and culture and correct diagnosis. The problem is then primarily one of prevention of anthrax bacteremia. Histologic examination of a pustule shows enormous numbers of bacilli in the lymphatic spaces with some walling off of leukocytes, but the zone of leukocytes is poorly developed, inasmuch as negative chemotaxis is exerted by toxic aggressins of the organisms.

Incision is dangerous and tends to break down the local defenses with consequent spread of the infection. When the lesion is large, in which case it may be mistaken for a carbuncle, simple incision is frequently followed by the development of bacteremia and death.

Destruction of the lesion with the *thermocautery* is one of the oldest treatments still widely practised in many parts of the world. When large lesions are removed it may lead to slow convalescence and the production of large scars. One other objection is that coagulation of the tissues interferes with subsequent drainage and free drainage is important for the prevention of the spread of the infection and more particularly of the dreaded generalized bacteremia.

The *local injection of germicides*, as phenol, iodine, bichlorid of mercury, zinc chlorid, and other substances, has long been a favorite treatment. The plan is to inject a germicide, 3 per cent. carbolic acid being mostly employed, at different points in the base of the pustule in order to destroy the bacilli, excite leukocytosis and liquefaction necrosis of the pustule. This treatment, however, is apt to prove very painful and due care must be exercised against the injection of toxic amounts of the germicide.

Excision of the lesion is widely practised. When thoroughly done including not only the pustule, but a portion of the edematous base, it removes at once the necrotic tissue and an enormous number of bacilli leaving a clean base for drainage. A drawback, however, is the resulting scar and deformity, although in the Philadelphia Hospital for Contagious

¹ Bull. méd., 1913, 1137.

² Lancet, 1904, 2, 372.

³ New York Med. Jour., July 21, 1917, 110.

⁴ Jour. Amer. Med. Assoc., 1918, 70, 671.

Diseases, where excision is commonly practised, I have seen many cases with extensive excision heal promptly without secondary pyogenic infection and with surprisingly slight scar formation and deformity.

The *local injection of antianthrax serum* has been advocated particularly by the Regans. "The method consists in the injection of 2, 2.5, or even 3 c.c. of serum at each of three or four points equidistant from one another at the various sides of the pustule. The needle is best inserted into the red indurated area of the pustule just beyond the blanched zone, the serum being directed toward the base of the eschar and injected so as to circumscribe the lesion. The injections are given once, twice, or three times in the twenty-four hours, depending on the severity of the case, and not more than 7 to 10 c.c. are injected at one time. Commonly four to six injections suffice."

The size of the lesion is not a reliable index of the chances for blood infection; in 1 case I observed a rapidly fatal bacteremia from a very small pustule developing in a hair follicle on the chest of a male worker in hides.

The following plan is advised for the systematic treatment of the malignant pustule, the various steps being given in consecutive order:

1. Give 1 c.c. of antianthrax or normal horse-serum subcutaneously for the purpose of desensitizing the patient in preparation for the intravenous injection of serum. This is not necessary if the large dose of serum is given subcutaneously or intramuscularly.

2. Under ether anesthesia excise the lesion, taking in a wide base and handling the lesion as little as possible, being particularly careful not to squeeze or handle it roughly. After excision and attention to hemostasis, the wound may be dusted with calomel and powdered ipecac and dressed with sterile gauze. Ipecac was advocated in 1888 by Muskett¹ and has been used in the Philadelphia Hospital for Contagious Diseases with considerable success. Both ipecac and its alkaloid emetin are anthracidal, the latter being studied by Smith and the writer.² Or the wound may be dressed with 1 : 1000 solution of bichlorid of mercury or mercuraphen in 10 per cent. sodium chlorid solution, the bichlorid or mercuraphen acting as a preventive for secondary pyogenic infection and an anthracidal agent, while the hypertonic saline excites a reversal of lymph flow into the wound and encourages drainage. Mercuraphen has been produced by Schamberg, Raiziss, and the author, and possesses an extremely high anthracidal activity even in the presence of blood and serum.

3. If, however, excision is refused or cannot be practised, the local injection of antianthrax serum after the method of Regan and described above, is advised. Furthermore, even if the lesion is excised, but the edema persists with signs of extension of the disease around the margins of the wound, serum should be injected locally as described.

4. The next step is to conduct a blood-culture, removing 5 to 10 c.c. of blood from a vein at the elbow into a flask containing 100 to 200 c.c. of nutrient broth. Special care should be exercised against contamination, because *Bacillus subtilis* may be mistaken for *B. anthracis*.

5. It is now advised to give an injection of antianthrax serum. If the lesion is quite small it will suffice to give 40 c.c. intramuscularly. If the lesion is large, with considerable edema and lymphadenitis, it is better to give 50 to 100 c.c. intravenously.

6. The blood-culture is closely watched. If anthrax bacteremia is present the bacilli will grow up within eighteen to twenty-four hours and indicate vigorous intravenous treatment. If the blood-culture is sterile

¹ Lancet, 1888, 1 269.

Jour. Infect. Dis., 1916, 18, 247.

at the end of twenty-four hours, serum need not be given, but it is advisable to repeat the blood-culture daily for the following two or three days.

Treatment of Anthrax Bacteremia and Internal Anthrax.—Antianthrax serum should be injected intravenously in dose of 100 to 200 c.c. as soon as possible. It is also a good plan to include an injection of neoarsphenamin, by reason of the reports indicating that arsphenamin and neoarsphenamin exert a curative influence.

My plan is to dissolve 0.9 gm. neoarsphenamin in 100 c.c. of sterile saline solution, add 100 c.c. of antianthrax serum, and inject the mixture intravenously.

Twelve hours later a second injection of serum alone should be given. Twelve hours later the injection of serum and neoarsphenamin may be repeated. Blood-cultures should be made daily and the injections of serum kept up until the blood is found to be sterile. If and when this fortunate result is obtained, at least one intramuscular injection of 30 to 50 c.c. of serum is advised as a precautionary measure.

If antianthrax serum is not available it is well to inject fresh sterile *beef serum*, which may be collected by bleeding from a jugular vein or an abattoir, blood being collected in a sterile vessel and the serum separated. Kraus, Penna and Cuenca¹ have treated anthrax in the Argentine Republic by injecting normal beef serum, previously heated twice at 56° C. for thirty minutes, in doses of 30 to 50 c.c. intramuscularly and repeating in twelve, twenty-four, or thirty-six hours, as the case may require. According to their latest report on 200 cases the mortality has been reduced to 0.5 per cent. as compared with 10 per cent. in 250 cases treated in the usual manner during the preceding ten years. Solari² and Langon³ have also reported favorably, but Lignieres⁴ states that beef serum is inferior to antianthrax serum.

Koehler, Wanner, and the writer⁵ found normal beef serum in this country only feebly anthracidal and without protective value for experimental infections in mice. However, since anthrax is widely prevalent among cattle in the Argentina, it is probable that beef serum there possesses higher curative values by reason of natural immunization of the cattle. A more probable explanation for the curative activity of normal beef serum is, however, that it excites a non-specific protein shock reaction of which the resulting leukocytosis is probably curative. At any rate the work of Kraus, Penna, and their associates indicates that normal beef serum is worthy of trial, although I believe that preference should be given antianthrax serum when it is obtainable.

THE SERUM TREATMENT OF PLAGUE

In so far as specific therapy is concerned the *prompt administration of antiplague serum in large doses and by intravenous injection* has proved of decided value especially in the treatment of the bubonic type of the disease; in the highly fatal pneumonic type it has also apparently saved some lives.

Antipest Serum.—The preparation of this serum is a difficult matter and different methods have been described.

The serum at present mostly employed is obtained from horses after repeated intravenous injections of killed cultures sometimes followed by

¹ Prensa Méd. Argentina, 1917, 3, 297; *ibid.*, 1917, 4, 91, 147; *ibid.*, 1918, 4, 455.

² *Semana méd.*, 1917, 24, 98.

³ Prensa Méd. Argentina, 1917, 4, 49, 370.

⁴ *An. de Facul. med.*, 1918, 3, 258.

⁵ *Jour. Infect. Dis.*, 1920, 26, 148.

living cultures after the method described for the preparation of anti-dysentery serum. It is bactericidal for the bacillus of pest and also contains agglutinins that aid in bacteriolysis and phagocytosis. Potent sera also contain opsonins, and a part of its protective and curative properties are ascribed to their presence. Some sera are feebly antitoxic for the small amounts of exogenous toxins produced by the organisms.

(a) In addition to Yersin's serum, which is prepared at the Pasteur Institute of Paris by immunizing horses with dead and then with living cultures of pest bacilli, other serums have been prepared. For example:

(b) Kolle immunizes horses with intravenous injections of heat-killed cultures, beginning with $\frac{1}{4}$ agar slant culture and doubling the dose each week until 15 cultures are given at one time. The horses are bled fourteen days after the last dose is given.

(c) Lustig immunizes horses with pest-nucleoproteins, obtained by breaking up the bacilli with 1 per cent. of potassium hydroxid and precipitating the proteins with acetic acid. These are then suspended in sterile normal salt solution, as in the preparation of Lustig's vaccine.

(d) Terni-Bandi immunizes donkeys and sheep with aggressins obtained by intraperitoneal injection of guinea-pigs with pest bacilli.

(e) Markl immunizes horses with filtrates of old pest bouillon cultures. He believes that the value of pest serum is largely dependent upon antitoxins.

The serums are usually tested by injecting mice with lethal doses of pest culture and decreasing doses of antiserum. The agglutinin content may also be measured. According to Strong pest immune sera do not contain appreciable amounts of bacteriolysin, but are largely bacteriotropic in action. Whenever cultures are used in immunization, the serum should always be cultured carefully and tested by animal inoculation to guard against the possibility of living bacilli being present.

Dosage and Administration of Serum.—A common mistake made in the serum treatment of this disease has been the administration of too small doses (10 to 20 c.c.) by subcutaneous injection. *Larger amounts of serum are required at the earliest possible time.* If intravenous injection is not possible, as may be the case in children, 40 to 80 c.c. of serum should be injected intramuscularly. Fontes has given the serum intraperitoneally to children and has published the following table showing the results:

METHOD.	CASES TREATED.	DIED.	DEATH-RATE.
Subcutaneous.....	21	8	38
Intraperitoneal.....	11	2	18
Intravenous.....	69	5	7.2

Chocky,¹ Moreno,² Guiteras and Ricio,³ and others have also advocated the intravenous injection of large doses of serum immediately upon the confirmation of the diagnosis.

The importance of early treatment is shown in the table published by Choksy in Bombay:

DAY OF THE DISEASE.	NUMBER OF CASES.	DIED.	RECOVERED.	MORTALITY, PER CENT.
First.....	323	98	225	30.3
Second.....	311	164	147	52.7
Third.....	248	155	93	62.5
Fourth.....	106	60	46	56.6
Fifth.....	52	32	20	61.5
Sixth.....	14	8	6	57.1
Seventh.....	4	4	0	100

¹ Brit. Med. Jour., 1908, 1, 1282.

² Series of seven papers in *Semana méd.*, 1915, 22, beginning with No. 30 and ending with No. 45.

³ Library and Press: La Moderna Poesia, Havana, 1915.

The following plan of serum treatment, slightly modified after that employed by Chosky, is recommended:

1. The intravenous injection of 80 to 100 c.c. of serum as soon as the diagnosis is made. For children under twelve years the dose may be 50 c.c. If an intravenous injection cannot be given a child, the serum may be injected intramuscularly or intraperitoneally.

2. Repeat the injection six hours later if the temperature has not fallen. If this has occurred the second injection may be given twelve to eighteen hours later.

3. The amount of serum to be injected subsequently will depend upon the rise of the temperature the previous evening, and the general condition of the patient. If the temperature be the same as on the first evening, the same amount may be injected; if it be lower, 30 c.c. or less should be injected.

4. The quantity of serum injected should be lessened gradually until the temperature falls to the normal in the mornings.

5. A sudden fall of the temperature between the second and the seventh day should not indicate a suspension of the treatment.

6. The injections are given every twelve hours if secondary buboes present themselves, or if the temperature rises rapidly one or two degrees.

7. If the evening temperature be lower than that of the morning, the dose is reduced on the following day.

8. From six to eight injections are generally sufficient to complete the treatment.

9. The total quantity of serum required varies between 150 and 300 c.c. according to the gravity of the symptoms and the condition of the serum.

Value of Serum Treatment of Plague.—When serum was first introduced by Yersin in 1896 the doses were small (10 to 20 c.c.) and usually administered by subcutaneous injection. Since then Yersin and others have employed larger doses, but the mortality statistics vary greatly owing not only to the use of sera of varying potencies, but to different doses and routes of administration and to the fact that plague epidemics vary greatly in virulence. The table shown on page 973, prepared by Dujardin-Beaumetz and quoted by Guiteras and Ricio, shows the wide fluctuations in mortality rates of plague treated with serum by different observers.

Chosky's mortality varied from 30 per cent. among cases treated on the first day to 100 per cent. treated on the seventh day, as shown in the table given above. His general mortality was 63.5 per cent. for 400 cases treated with serum as compared with a mortality of 74 per cent. among 200 cases treated without serum. Burnett¹ reported a mortality of 29.7 per cent. among serum treated cases as against 73.9 per cent. treated without serum. Penna in Argentina, reports that among 664 cases treated with Yersin's serum during the period 1905 to 1912, the mortality ranged from 23 per cent. in 1906 to 7.3 per cent. in 1912, with an average mortality of 12.5. From 1914 to the middle of 1919 Kraus' serum was used with an average mortality of 7.8. per cent. While the Plague Commission of India² did not report very favorably upon the use of Yersin's or Lustig's sera in 1913, later statistics indicate an improvement in the mortality rates ascribed to the use of more potent sera in larger doses and by intravenous injection.

¹ Report on Plague in Queensland, 1900-07, Brisbane, 1907.

² British Commission, Seventh Report on Plague Investigation in India, Jour. Hyg., Supplement II, 1913, 326.

AUTHOR.	EPIDEMIC.	TREATED.	DIED.	MORTALITY, PER CENT.
	Canton and Amoy, 1896.....	26	2	7.6
Yersin.....	Bombay, 1897.....	50	17	34.0
	Nha-Trang, 1898.....	33	14	42.4
	Mandvi, 1898.....	136	89	65.4
Simond.....	Bombay, Karad, Moundra, 1898...	171	99	57.8
	Kuratchi, 1898.....	75	37	49.3
Zabolotny.....	Mongolia, 1898.....	16	12	75.0
Delay.....	Mongtzé, 1898.....	10	4	40.0
Thiroux.....	Tamatave, 1898-99.....	20	11	55.0
Calmette and Salimbeni..	Oporto, 1899.....	142	21	14.7
Métin.....	Oporto, 1899.....	6	1	15.6
Primet.....	Noumea, 1899-1900.....	7	2	28.5
Noc.....	Noumea, 1901.....	17	8	46.9
Auber.....	Réunion, 1899.....	8	1	12.5
Vassal.....	Réunion, 1900-01.....	13	2	15.3
Clarac and Manguy.....	Majunga, 1902.....	71	32	45.0
Roufiandis.....	Foa-Tchéou, 1902.....	67	34	50.7
	Tonkin, 1903.....	101	51	50.5
Choksy.....	Bombay.....	51	37	72.5
Agote and Medina.....	Rosario.....	26	11	42.3
Penna.....	Buenos Aires, 1905.....	204	29	19.3
Del Rio and Zegers.....	Iniique, 1903.....	85	38	44.7
Montero.....	Autofogasta.....	50	3	6.0
Cruzat.....	Chanarai, 1904.....	18	1	5.5
Godinho.....	Santos, 1900.....	19	7	36.8
Duprat.....	Rio Grande, 1902.....	45	7	15.5
	Rio Janeiro, 1900.....	410	138	33.6
	Rio Janeiro, 1901.....	278	99	35.6
	Rio Janeiro, 1902.....	268	68	25.3
	Rio Janeiro, 1903.....	541	124	22.9
	Rio Janeiro, 1904.....	504	103	20.4
	Rio Janeiro, 1905.....	149	25	16.5
	Rio Janeiro, 1906.....	187	54	28.8
	Campos, 1902.....	136	27	19.1
Tavares de Marced6.....	Campos, 1906.....	14	2	14.2
A. Ferrari.....	Rio Janeiro, intravenous, 1907.....	69	5	7.2

TREATMENT OF ASIATIC CHOLERA

Numerous attempts have been made to develop an efficient serum therapy for this disease.

Anticholera Serum.—In some respects cholera would seem to be due mainly to a toxin elaborated by the bacilli in the intestinal tract of infected persons, similar to the action of the toxin of the Kruse-Shiga type of dysentery bacillus. Various attempts have been made to prepare an efficient anticholera serum, but the only one that has yielded encouraging results in experimental infections as well as in cholera of human beings is that prepared by Kraus. This serum is prepared by immunizing horses with a true toxin derived from a cholera-like vibrio isolated by Gottschlich from the intestinal contents of pilgrims dying at El Tor from a dysentery or cholera-like infection. According to Kraus, this antiserum is largely antitoxic, and serves to neutralize the toxin of true cholera more effectively than does the antiserum resulting from immunization with cholera cultures.

Antisera have also been prepared by immunization of horses with ground organisms in order to render it anti-endotoxic; also with killed and living cultures of the vibrio and extracts of these, by Carrière and Tomarkin.¹

¹ Ztschr. f. Immunitätsf., orig., 1909, 4, 30.

These antisera in addition to being antitoxic for exogenous and endogenous toxins of virulent cultures, are likewise bactericidal, agglutinative, and opsonic; their value in the treatment of the disease probably depends upon a combination of these activities.

Value of Anticholera Serum.—The results have not been very good. Doubtless the mortality rates have been influenced not only by the varying severity of different epidemics, but likewise by the use of sera of varying potencies and more especially by the doses employed and route of administration.

In most instances the serum has been given by subcutaneous injection. In the Russian epidemic of 1908–09 Stühlern¹ employed large doses by intravenous and subcutaneous injection combined with saline infusion; the best mortality rate was about 30 per cent. Slightly better results were reported by Salimbeni² in the treatment of 42 cases. Best results were obtained with the sera prepared by Schurupoff, and especially with that prepared by Carrière and Tomarkin.³ Jegunoff⁴ treated 12 patients with intravenous injections of Kraus' serum diluted with 500 to 700 c.c. of saline solution, with a mortality of 25 per cent. as compared with a general mortality of 75 per cent. in cases receiving no serum. Hundogger⁵ has reported unfavorably upon the use of the serum; Strong⁶ has thoroughly reviewed the literature up to 1907 and reports that the results have not been very encouraging.

Ketscher and Kering used Kraus' antitoxic serum in 119 cases, with a mortality of 58 per cent., among those treated with subcutaneous injections and 50 per cent. among those treated intravenously; the mortality among those not treated with serum was 63.4 per cent. Von Stühlern and Tuschinski observed a mortality of 29.9 per cent. among serum-treated cases.

More recently Livierato⁷ has reported the successful use of anticholera serum injected intravenously along with injections of hypertonic saline solution according to Rogers, and believes that the serum has an unmistakable antitoxic action and effectually supplements treatment with saline solution. It may be stated that in general terms the mortality of severe cholera has been reduced from 70 per cent. to about 25 per cent. when serum has been intravenously injected in large doses; doubtless a part of these beneficial results are to be ascribed to the replenishment of the blood volume, and best results are to be secured by a combination of serum and saline injections given intravenously.

Dosage and Administration.—Anticholera serum should be administered as early in the disease as possible and preferably by intravenous injection. A good plan is that employed by Jegunoff, who administered 100 to 150 c.c. of serum diluted with 500 to 700 c.c. of saline solution. The intravenous injection of saline solution alone has proved of great benefit, and especially during the first few days of the disease, to counteract the depletion of the tissues by diarrhea and vomiting.

Rogers employs a hypertonic saline solution prepared by dissolving 16 gm. sodium chlorid, 0.5 gm. calcium chlorid, and 0.74 gm. potassium

¹ Med. Klinik, 1909, 5, 1452.

² Ann. de l'Pasteur Inst., 1908, 22, 172; *ibid.*, 1910, 24, 34.

³ Ztschr. f. Immunitätsf., 1909, 4, 30.

⁴ Wien. klin. Wchn., 1909, No. 24.

⁵ Wien. klin. Wchn., 1909, No. 52.

⁶ Bull. No. 16, Bureau of Govt. Lab., Manila, 1904; *ibid.*, No. 21; Philippine Jour. Sci., 1906, 1, 501; *ibid.*, 1907, 2, 413.

⁷ Riforma med., 1915, 31, 673.

chlorid in 1000 c.c. of sterile distilled water. From 1000 to 2000 c.c. are injected intravenously, aiming to restore the blood-pressure to 110 mm. Hg.

The dose of serum should be repeated at intervals of twelve to eighteen hours until improvement is apparent. In children the injections may be given intramuscularly if intravenous injections cannot be given, but better results are likely to follow the intraperitoneal injection of serum (50 to 100 c.c.) and saline solution (500 c.c.) under these conditions.

Vaccine Treatment of Cholera.—The disease has not until recently been treated with vaccine owing to its very rapid course. During 1915, Petrovitch¹ employed a vaccine for the treatment of 1153 mild cases, with a mortality of 2 cases. In 90 cases of moderate severity none died, while the mortality was 9.4 per cent in a similar group not treated with vaccine. In 157 severe cases the mortality was 14.4 per cent., while of 120 similar control cases the mortality was 58 per cent. Saline infusions were also employed.

TREATMENT OF PERTUSSIS

Serum Treatment.—Antipertussis sera have not been prepared on a large scale by the immunization of horses with the bacillus of Bordet and Gengou. Serum treatment has been confined to the use of human serum or blood from normal persons and convalescent cases of the disease.

Bleyer² treated 45 cases during the early weeks of the disease, with intramuscular injections of compatible blood taken from donors within three months of recovery, from donors who had had the disease at more remote periods, and from donors who had never had pertussis. The dose varied from 40 to 125 c.c., divided into two, three, or four doses injected into the gluteal muscles. Blood not used at once was citrated to 1 per cent. Bleyer found that a few cases were apparently benefitted by the injections, but was not able to ascribe any of these effects to a specific action of the injected blood.

Pentz³ reports that the intramuscular injection of 3 children with convalescent serum had an apparent good effect in the way of mitigating the severity of the infection.

Vaccine Treatment.—Graham⁴ was first to use pertussis vaccine in the treatment of the disease, injecting 20,000,000 bacilli every four days and later increasing the dosage to 40,000,000. Twenty-four cases were treated and 17, or 71 per cent., were apparently benefitted. Since then a large literature has accumulated on the use of vaccines for the prophylaxis and treatment of whooping-cough. The vaccines employed have not always been prepared of *Bacillus pertussis* alone; in some instances mixed vaccines, including *B. influenza*, streptococci, and other organisms, have been included.

Sill⁵ reports 33 cases treated with vaccine. In all the number and severity of the attacks were markedly diminished. He advises 50,000,000 every second day in moderately severe cases and 100,000,000 in very severe cases. He reports 13 additional cases and 3 prophylactic cases which were exposed and proved to be immune. All 16 cases were treated with mixed pertussis vaccine: *Bacillus pertussis*, 50,000,000; *Staphylococcus aureus*, 20,000,000; *micrococcus catarrhalis*, 20,000,000. With best drug treatment the cough persisted from seven to twelve weeks; with pertussis vaccine, from four to four and half weeks. Of the last 10 treated with combined vaccine, all were cured in three and a half weeks.

¹ Bull. de l'Acad. de méd., 1915, 74, No. 33.

² Amer. Jour. Med. Sci., 1917, 154, 39.

³ Nederl. Tijdschr. v. Geneesk., 1917, 1, 714.

⁴ Tr. Amer. Pediat. Soc., 1911, 23, 157; Amer. Jour. Dis. Child., 1912, 3, 41.

⁵ Amer. Jour. Dis. Child., 1913, 5, 379.

Sill cites Dr. Freeman, of London, who treated 1140 cases with vaccine, with the following results: Much better, vaccine, 31 per cent.; control saline, 21 per cent. Better, 37.1 per cent.; control saline, 34.5 per cent. Unchanged, 15 per cent.; control saline, 23.9 per cent. Worse, 15.2 per cent.; control saline, 18.15 per cent. Much worse, 1 per cent.; control saline, 1.85 per cent.

Sill believes that no bad effects follow the vaccine treatment; that the average length of the cough under this treatment is about four and one-half weeks. He suggests giving the vaccine treatment every day for a few days in very severe cases and he is guided more by the severity of the disease than the age of the child. The cases seen earlier in the disease before the height of the paroxysms is reached respond most quickly to treatment. The younger child receives relatively larger doses, and responds more quickly to treatment. He considers this important because of the grave danger of pneumonia developing in the young child. He also believes that vaccine may confer immunity.

Nicolle and Conor¹ used living cultures of the Bordet bacillus, injected under the skin, every two or three days. The treatment was given to 122 children, but 18 were not seen after the first injection. Of the 104 that were treated, 37 were cured, 40 improved, and 27 remained stationary. In the cases cured the improvement was manifest early. The improvement was noticed after the first or second injection, the number and severity of the paroxysms being diminished.

Saunders, Johnson, White and Zahorsky,² Ladd,³ Scott,⁴ Wilson,⁵ Luttin-ger,⁶ Meyers,⁷ Bamberger,⁸ Hartshorn and Moeller,⁹ Huenekens,¹⁰ Polozker,¹¹ Shaw,¹² Reynolds,¹³ Bloom,¹⁴ Rewalt,¹⁵ Davies,¹⁶ and others, have reported favorable results from the treatment of pertussis especially when the injections were given early in the disease.

Bacher and Menschikoff,¹⁷ Hess,¹⁸ von Sholly, Blum and Smith,¹⁹ Barenberg,²⁰ and others did not find the vaccine of any particular value in treatment, although the consensus of opinion is to the effect that the vaccine when *freshly prepared* and given *early* in the disease in *appropriate doses* is of distinct value. Bloom states that vaccine treatment tends to minimize loss in weight, reduces the duration of the disease, decreases the severity of the disease, and reduces the percentage of complications and mortality.

Dosage and Administration of Pertussis Vaccine.—Stock vaccines are generally employed. These may be prepared of different strains of *B. pertussis* alone or in combination with pneumococci, streptococci, and other organisms.

The author employs a stock vaccine of several strains of *B. pertussis* with different strains of pneumococci in order to obtain some degree of

¹ Acad. d. Sci., 1913.

² Pediatrics, 1912, 24, 161.

³ Arch. Pediat., 1912, 29.

⁴ New York Med. Jour., 1913, xcvi, 176.

⁵ New York Med. Jour., 1913, xcvi, 823.

⁶ Med. Record, 1913, lxxxiv, 1125; Jour. Amer. Med. Assoc., 1917, 68, 1461.

⁷ Pediatrics, 1914, 26, 450.

⁸ Amer. Jour. Dis. Child., 1913, 5, 33.

⁹ Arch. Pediat., 1914, 31, No. 8 (gives a review of the literature covering 1445 cases).

¹⁰ Amer. Jour. Dis. Child., 1918, 17, 29.

¹¹ Amer. Jour. Obstet., 1916, 73, 551.

¹² Amer. Jour. Obstet., 1917, 76, 161.

¹³ Arch. Pediat., 1919, 36, 290.

¹⁴ Arch. Pediat., 1919, 36, 1.

¹⁵ Penna. Med. Jour., 1921, 24, 404.

¹⁶ Amer. Jour. Dis. Child., 1922, 23, 423.

¹⁷ Centralb. f. Bakteriolog., 1912, 61, 218.

¹⁸ Amer. Jour. Obstet., 1914, lxx, 510.

¹⁹ Jour. Amer. Med. Assoc., 1917, 68, 1451.

²⁰ Amer. Jour. Dis. Child., 1918, 16, 23.

prophylactic immunization against pneumococcus pneumonia at the same time. This vaccine is prepared of 400,000,000 of *B. pertussis* and an equal number of each of the four types of pneumococci per cubic centimeter, yielding a vaccine containing a total of 2,000,000,000 per cubic centimeter. For children under five years of age the dose is 0.1 to 0.2 c.c. (200,000,000 to 400,000,000) every other day by subcutaneous injection; for children five to twelve years the dose is 0.5 to 1 c.c. every three to four days by subcutaneous injection.

After the paroxysmal stage has been passed, the writer employs an autogenous vaccine for the treatment of the bronchitis and residual infection. This is usually prepared of staphylococci, streptococci, and pneumococci depending upon the bacteriologic findings after plating sputum on blood-agar plates. The vaccine is made up of equal proportions of the different bacteria, each cubic centimeter containing 2,000,000,000. The doses begin with 0.1 c.c. and are gradually increased at intervals of four to six days.

TREATMENT OF GONOCOCCUS INFECTIONS

Immunity in Gonococcus Infections.—Human beings only are naturally susceptible to infection by the gonococcus, although the urethral and conjunctival mucous membranes of some of the apes have been experimentally infected with virulent cultures with the production of slight and rapidly disappearing inflammatory changes resembling gonococcus urethritis and conjunctivitis of human beings. Other lower animals, as rabbits and guinea-pigs, may be intoxicated by the intraperitoneal and subcutaneous injection of virulent gonococci and autolysates, but the mucous membranes resist infection when the gonococcus is applied in culture or in pus.

Little is known of the nature of natural immunity to the gonococcus in human beings. The organism appears to possess a selective affinity for certain tissues, notably the mucous membranes of the urethra and adnexa of both sexes, the eye (conjunctival mucosa and iris), endocardium, and synovial membranes. Infections of the nasal and oval cavities are very rare; the rectum is sometimes infected and the meninges and other interval organs are very rarely attacked. The mucous membranes of the young are more susceptible than adult tissues; the conjunctival mucosa of babies and the mucosa and skin of the urogenital system of female children are especially susceptible, whereas the conjunctival mucosa of adults apparently acquires natural resistance in view of the relative infrequency of involvement considering the opportunities for infection.

Serologic strains of gonococci probably exist analogous to the different types of meningococci. The investigations of Louisa Pearce¹ indicate that the gonococci producing infantile vaginitis may be different serologically from those producing adult infections. Torrey and Buckell² have recently studied the problem in a most exhaustive manner and found on the basis of agglutinin absorption tests, that it was not possible to group gonococci in distinct immunologic types. However, they were able to classify their strains under three general headings of (a) regular, (b) intermediate, and (c) irregular strains. No definite serologic distinction could be drawn between strains isolated from vulvovaginitis cases in children and those from gonorrheal infections in adults. There appears to be abundant clinical evidence to the effect that the gonococci from urogenital infections of adults of both sexes may produce gonococcus infection of the urogenital and other mucous membranes of children of both sexes; the

¹ Jour. Exper. Med., 1915, 21, 289.

² Jour. Immunology, 1922, 7, 305.

evidence is much less conclusive that gonococci from infantile infections are able to excite the disease in adults, although this is probably the case.

The great majority of human beings are apparently susceptible to gonococcus infection; the escape of individuals in a group exposed to infection is to be ascribed more to fortuitous circumstances than to individual resistance. However, a natural local or systemic immunity has been observed by Hess¹ in a small number of female children who have escaped infection when exposed to cases of gonococcus vaginitis one or more times.

Acquired immunity to the gonococcus never reaches a high degree. An individual recovering from gonorrhea is susceptible to reinfection. Probably the latency of chronic infections is due in part to the production of immunity principles retarding the activity of the organisms; it is known, however, that these gonococci are able to infect others and that, in turn, these may superinfect the original host. It would appear, therefore, that the tendency to latency of gonococcus disease in both sexes and the spontaneous cure of vaginitis of children as they approach puberty are to be ascribed more to tissue changes, alteration of secretions, and reduction of virulence of the organisms than to the acquisition of curative and protective immunity principles.

Resistance to gonococcus infection and recovery from infection is largely a function of phagocytosis, although there is abundant evidence to indicate that the gonococcus enjoys a peculiar resistance to the destructive activities of the endolysins (cytases) of leukocytes, and that the leukocytes in turn are relatively slightly harmed by the gonococci. Gonococci produce toxins, largely endogenous, which are regarded as the principle pyogenic substances.

In chronic and wide-spread gonococcus disease various specific antibodies, as opsonins, bacteriolysins, agglutinins, precipitins, and complement-fixing antibodies, may be found in the blood, but of these, the opsonins are of most importance. The gonococcus, however, is not actively antigenic, that is, is not very active in stimulating the antibody-producing tissues. For this reason antibody production in ordinary gonococcus infection does not reach a high degree, active prophylactic immunization against the disease has not been accomplished, vaccine treatment has not proved generally successful, and the immunization of horses and other animals for the production of immune sera is prolonged and seldom results in the production of highly potent immune sera.

Antigonococcus Serum in the Treatment of Arthritis; Acute Epididymitis; Prostatitis and Orchitis; Gonococcus Septicemia.—Antigonococcus serum is prepared by the immunization of goats and horses with different strains of gonococci and was first described by Torrey and Rogers² in 1906.

Torrey's serum is prepared by immunizing rams with gradually increasing intraperitoneal doses of dead, and later of living, cultures of gonococci. Larger amounts of serum may be secured by immunizing horses according to the methods described for the preparation of meningococcus and streptococcus immune serums, and in view of the larger doses now advocated, this is advisable. This serum has been successfully concentrated in the same manner as is diphtheria antitoxin by Heinemann and Gatewood.³

According to Torrey, antigonococcus serum is largely bactericidal in nature. The presence of antitoxins has not been demonstrated, but potent sera are also capable in some degree of neutralizing the endotoxins liberated or formed when the cocci are broken up. The serum also contains agglu-

¹ Amer. Jour. Dis. Child., 1916, 12, 466.

² Jour. Amer. Med. Assoc., 1906, xlvii, 261, 273.

³ Jour. Infect. Dis., 1912, 10, 259.

tinins, complement-fixing antibodies, and opsonins, and its curative properties are probably due to the activities of these antibodies in addition to the production of fever, leukocytosis, and other non-specific agencies.

Antigonococcus serum has been employed to some extent in the treatment of ordinary *acute gonorrhea* of the male and female, but only in doses of 2 to 3 c.c. by subcutaneous injection which were insufficient for bringing into play specific and non-specific agencies. There is evidence indicating that fever of 101° to 103° F. is curative in gonococcus infections, inasmuch as the gonococcus is highly susceptible to heat, and rapid improvement has been noted by some observers when the serum was given intravenously followed by a febrile reaction and leukocytosis. These effects are purely non-specific, and since the reaction is unpleasant, serum therapy is not employed in the treatment of ordinary acute gonorrhea.

In the treatment of acute gonorrheal complications, however, antigenococcus serum is sometimes useful and especially in severe fulminating *epididymitis*, *prostatitis*, and *orchitis*, *salpingitis*, *gonococcus bacteremia* (*sepsis*), and metastatic lesions, especially *arthritis*. This is particularly true when the serum is given early, intravenously or intramuscularly, and in amounts of at least 30 to 50 c.c. instead of 2 to 3 c.c. by subcutaneous injection. *Gonococcus bacteremia* is not commonly detected, but when the condition is found it may be stated without reserve that the intravenous administration of the serum is indicated in order to rid the blood of the cocci and probably prevent the involvement of other mucous membranes, as the joints and endocardium. Given early in *arthritis*, upon the first evidences of inflammation, it may abort the infection. After the arthritis is well developed, however, the serum may bring about only temporary relief, and equally good results have been observed following the intravenous injection of normal horse-serum and other protein agents, eliciting non-specific reactions. That antigenococcus serum is useful in the treatment of arthritis is indicated by the reports of Uhle and MacKinney,¹ Herbst and Belfield,² Swinburne,³ Schmidt,⁴ Corbus,⁵ and others.

In acute gonococcus infections antigenococcus serum is, therefore, indicated and probably useful, and particularly when the infection is especially acute and wide-spread, with metastases to neighboring or distant tissues. If serum is given at all it should be given in adequate doses, as stated above. A second injection should follow in about twelve hours and probably a third on the second day, until there are evidences of improvement. Debré and Paraf⁶ have recently reported the successful treatment of 15 cases of acute gonococcus arthritis by the injection of antigenococcus serum directly into the joints at intervals of one to three days, followed by the application of compressing dressings. At the same time serum is given intramuscularly or intravenously.

Autoserum therapy has been occasionally practised. Wagon⁷ reports that in 4 cases of *orchitis* with effusion the removal of the fluid and subcutaneous injection of 1 c.c. was followed by good results. It is highly probable, however, that the tapping alone was the more beneficial of the two procedures.

Dufour and Debray⁸ have also treated arthritis by the same method, aspirating fluid from the larger joints and reinjecting it without heating. In 3 cases the general symptoms and local pain rapidly subsided.

¹ Jour. Amer. Med. Assoc., 1908, 41, 105.

² Illinois Med. Jour., 1908, 13, 689.

³ Trans. Amer. Urol. Assoc., 1909, 3, 170.

⁴ Therap. Gaz., 1909, 26, 609.

⁵ Jour. Amer. Med. Assoc., 1914, 62, 1462.

⁶ Bull. d. l. Soc. méd. d. hôp., 1919, 43, 908.

⁷ Bull. de l'Acad. de méd., 1916, 76, 81.

⁸ Bull. d. l. Soc. méd. d. hôp., 1920, 44, 1399.

Subcutaneous Injections of Gonococcus and Mixed Vaccines in the Treatment of Acute Urethritis.—Gonococcus vaccines injected subcutaneously in ordinary doses have been used extensively in the treatment of *acute gonorrheal urethritis* of the male and female with a wide divergence of opinion in regard to their value. Some physicians believe that their administration is useful for shortening the disease and preventing extensions and complications, while others, and probably the majority, believe that they are useless, as recently stated by Geraghty.¹

Boyd² has treated 250 cases with vaccines prepared of gonococci and staphylococci, the latter isolated from cases of mixed infection, and concludes that there is no evidence indicating that this mixed vaccine in ordinary or in large doses had any effect upon the course of early acute cases of gonorrhea in men.

Lumb,³ however, believes that vaccine treatment results in a material reduction in the duration of the disease and that relapses occurred in less than 1 per cent. of a series of cases. Baril and Creuze⁴ and Gibson⁵ have also advocated vaccines in acute and chronic gonorrhea for the purpose of building up general resistance and preventing chronic complications.

Stock vaccines are generally employed and in larger doses than usual; injections are made subcutaneously every two or three days, beginning with at least 100,000,000 cocci and gradually increasing. Slight reactions are apparently necessary for therapeutic results.

The cultivation of the gonococcus is not particularly difficult during the acute stages of gonorrhea and the autogenous strain may be secured, prepared in a vaccine, and held in reserve for later use if the infection becomes chronic with involvement of neighboring organs.

Subcutaneous Injections of Gonococcus and Mixed Vaccines in the Treatment of Chronic Urethroph prostatitis, Epididymo-orchitis, and Arthritis.—These chronic infections so difficult of cure have received special attention from the standpoint of vaccine therapy. In the treatment of chronic urethritis and the extensions, mixed stock vaccines of gonococci, staphylococci, colon bacilli, and diphtheroid bacilli have been generally employed.

Given by subcutaneous injection and in ordinary doses at intervals of five to seven days it may be stated that in the opinion of many physicians the results have been frequently negative, that is, beneficial effects have not been observed in the majority of cases.

It is apparent, as recently stated by Sézary,⁶ that the doses must be larger than usually given in order to secure a febrile reaction and a focal reaction (increased discharge, etc.) before beneficial effects are to be obtained. For this purpose the writer prepares an autogenous vaccine of staphylococci and other bacteria secured in cultures of secretion after prostatic massage, and adds a stock polyvalent vaccine of gonococci. Each cubic centimeter contains 2,000,000,000 (1,000,000,000 gonococci and 500,000,000 of each of the other organisms); the injections are given intramuscularly beginning with 0.2 c.c. (200,000,000) and rapidly increased at intervals of five to seven days. Used in conjunction with local measures many cases are apparently benefited; at least the focal reactions combined with prostatic massage appear to throw off large numbers of organisms with gradual disappearance of urethral discharge.

¹ Jour. Amer. Med. Assoc., 1921, 76, 35.

² Jour. Royal Army Med. Corp., London, June, 1921.

³ Brit. Med. Jour., October 6, 1917, 450.

⁴ Paris Med., 1919, 9, 202.

⁵ Lancet, 1919, 2, 739.

⁶ Progrès méd., Paris, May 14, 1921.

In *arthritis*, vaccines of gonococci administered by subcutaneous injection have sometimes proved useful adjuvants in treatment. Heaworth¹ has reported favorably on the use of sensitized vaccine. It would appear that in the past the doses have been too small. Better effects are to be secured with doses large enough to produce a general reaction of fever associated with some degree of focal reaction in the joints. For these purposes intramuscular injections are better than subcutaneous injections.

Best results have been secured by intravenous injections, which elicit well-marked non-specific general reactions; this treatment will be described later, but should be employed very cautiously by reason of the possibility of eliciting too severe reactions.

Pearson² has recently advocated the *subcutaneous injection of gonococcus vaccine as a provocative measure to determine cure of urethritis*. In none of his cases did the vaccine produce a general reaction nor was any discomfort caused to the patient and no complications followed its use. On the morning of the first day a dose of 3,000,000 of polyvalent gonococcal vaccine is administered subcutaneously, and at the same time the seminal vesicles, prostate, and Cowper's glands are massaged thoroughly to liberate any toxins confined in them. The patient is then instructed to hold his urine from midnight of that night until a smear is taken the next morning, and to do the same for each successive night until four smears have been obtained. On the morning of the second day a dose of 5,000,000 of the vaccine is administered. Should the case still be infected with the gonococcus, a positive smear may be obtained on one of the four mornings. Should there be no infection with the gonococcus, the smear will remain negative for that organism. Of 100 consecutive cases the provocative vaccine gave a reliable result in 96 per cent. of cases, but Pearson advises that it should not be relied on solely; it should be used in conjunction with other routine tests. Its use renders unnecessary the irritation of the urethra produced by strong provocative injections of chemicals.

Subcutaneous Injections of Gonococcus Vaccine in the Treatment of Vaginitis and Cervicitis.—A large literature has accumulated on the results of the vaccine treatment of vulvovaginitis and cervicitis and especially of children. The reports are contradictory, but the results have been generally negative.

Hess has reported that subcutaneous injections of 100,000,000, 200,000,000, or 400,000,000 of killed cocci sometimes elicits a focal reaction of increased vaginal discharge of pus cells and gonococci in cases of chronic and latent infections of children, and that one or two doses have proved useful for diagnosis by reason of these provocative reactions. Hess also believes that the injections have some prophylactic value when used routinely among female children prior to admission in the wards of a hospital where gonococcus vaginitis may make its appearance.

The disease in both children and adults soon involves the cervix, almost invariably becomes chronic, and proves extremely difficult of cure. It would appear that the administration of vaccine in amounts large enough to elicit a focal reaction may be useful as an adjuvant to local treatment. Doses less than required to produce hyperemia and increased discharge (focal reaction) have been proved to be useless.

Intravenous Vaccine and Non-specific Agents in Arthritis, Prostatitis, and Epididymitis.—Bruck and Sommer³ have reported exceptionally good results in the treatment of acute and subacute gonococcus arthritis, prosta-

¹ Brit. Med. Jour., 1918, 1, 4.

² Jour. Urology, 1918, 2, 455.

³ Münch. med. Wchn., 1913, 60, 1185.

titis and epididymitis by the intravenous injection of polyvalent gonococcus vaccine. Culver¹ has injected intravenously 100,000,000 killed gonococci in the treatment of these infections, giving the same or slightly different amounts every four or five days, until about five injections had been given.

"The injection is followed by a chill of variable severity coming on in from twenty minutes to one hour and lasting from fifteen to thirty minutes. This chill may or may not be accompanied by headache, which is usually of short duration and passes away shortly after the chill is over. Exceptionally there are nausea and vomiting during the first few hours, but it is never severe and is always transient. This occurs always in patients who have disobeyed instructions by eating heartily within a few hours of the injection.

"At the onset and during the chill the patient often complains of severe pain in the affected parts. Whether this is due to some internal cause or to the unavoidable motion produced by the chill it is impossible to say. The pain, however, is variable in different patients and in the same patient at different times.

"It is usual for the severity of the reaction to decrease following repeated injections, and this decrease is directly proportional to the number of injections previously given in order to maintain a constant reaction with each succeeding injection of gonococcal gradually and cautiously increased.

"The leukocyte counts during a reaction and following it are somewhat variable. Usually there is a mild leukocytosis just before and during the first part of the chill, soon followed by a marked leukopenia, which appears toward the end of the chill. A count as low as 2000 has been observed repeatedly during this stage of the reaction. This condition is soon followed by a gradually developing leukocytosis which reaches its maximum in from five to seven hours, and remains moderately high for from twenty-four to thirty hours. A return to normal occurs in about forty-eight hours."

Similar reactions and clinical results were observed after the intravenous injection of a vaccine of 100,000,000 meningococci as well as after the injection of 25,000,000 colon bacilli and 2 c.c. of a 4 per cent. solution of secondary proteose made from casein. For these reasons Culver ascribed the effects and beneficial results to non-specific reactions embracing the febrile reaction, leukocytosis, mobilization of ferments, and to some extent an increase of gonococcus antibodies.

"Twenty-four patients suffering from *arthritis* associated with gonorrheal urethritis were treated. Naturally, most of these cases were acute or sub-acute, but some were of five months' duration and many were of over ten weeks' duration when the treatment was begun.

"As might be expected, the most striking results were obtained in the acute and subacute cases; the most refractory cases, however, were also in the acute class. Those suffering for long periods appear to respond more slowly to the treatment, but fortunately seem to suffer from no recurrences or new joint involvements during the course of the treatment, as some of the more acute cases do. All but two of the arthritic patients were completely cured or manifested a decided improvement. The length of treatment varied from two days to one month.

"Unusual effects were seen in three patients with acute arthritis so severe that sedatives were necessary to give them rest for the first two days in the hospital. After a single reacting dose in each instance they felt so well that they insisted on getting out of bed, and in three days they walked out of the hospital. Two of the patients had effusions in the knee-joint, which completely disappeared before their discharge from the hospital.

¹ Jour. Amer. Med. Assoc., 1917, 68, 362; Jour. Lab. and Clin. Med., 1917, 3, 11.

"Twelve patients with *acute epididymitis* were treated, and invariably the pain would subside after the first injection. Usually not more than two injections were necessary and, indeed, in most instances one, to effect a cure. The swelling begins to subside within twenty-four hours after the first injection."

In a subsequent review of this work Culver¹ states that in his experience at one of the military camps during the war where intravenous injections of gonococcus protein were made in every case of epididymitis, the results were satisfactory, the average stay in the hospital being from five to six days. Even the primary localization of the cocci in the urethra or extensions of the infection into the prostate and seminal vesicles responded more readily to local treatment after these injections than cases not injected.

Miller and Lusk² have likewise observed encouraging results in the treatment of acute and chronic gonorrheal arthritis from intravenous injections of typhoid vaccine (100,000,000); Cecil,³ however, noted but little improvement after the intravenous injection of typhoid and gonococcus vaccines in arthritis. Luithlen⁴ has had considerable and favorable experience in arthritis treated with intravenous injections of 100,000,000 of gonococci; he recommends, though, that local treatment should not be neglected.

Block⁵ has also employed intravenous injections of gonococcus and typhoid vaccine and obtained the best clinical results when well marked reactions were produced, particularly a sharp rise in temperature.

Müller and Weiss⁶ have reported very favorably upon the therapeutic effects of intramuscular injections at intervals of four to five days, of 5 to 10 c.c. of market *milk*, boiled for ten minutes and cooled, in the treatment of acute and subacute gonococcus arthritis, prostatitis, and orchitis. Konteschweller⁷ also reports very satisfactory results in the treatment of gonococcus arthritis from the intramuscular injection of milk and kefir and the intravenous injection of peptone. Trossarello,⁸ however, did not observe any beneficial results in arthritis from milk injections.

Weiss⁹ has treated a number of cases of epididymitis by injecting 5 to 10 c.c. of sterile milk in the scrotal skin and claims excellent results.

Smith¹⁰ has observed that patients suffering from gonorrheal complications are frequently benefited when decided general reactions follow the intravenous injection of *normal horse-serum* as well as horse antigonococcus serum; Brown¹¹ has also reported the beneficial effects following the injection of normal horse-serum and diphtheria antitoxin in the treatment of epididymitis. These results are purely non-specific effects. Müller¹² and Saudek¹³ have also treated with success gonorrheal complication by the injection of 1 to 2 c.c. of sterile normal horse-serum about the site of the lesion.

Subcutaneous injections of *turpentine* have been employed by several observers and especially Krebs¹⁴ in the treatment of both acute and chronic gonococcus infections. Krebs has treated several hundred cases with injections every three to five days combined with appropriate local treatment. He claims good results and particularly in the severer infections; the acute disease was shortened and complications lessened.

These results indicate that non-specific therapy has proved useful in the

¹ Jour. Amer. Med. Assoc., 1920, 76, 311.

² Jour. Amer. Med. Assoc., 1916, 68, 2010.

³ Arch. Int. Med., 1917, 20, 951.

⁴ Wien. klin. Wchn., 1919, 32, 448.

⁵ Cor.-Bl. f. schweiz. Aerzte, 1914, xlv, 1377.

⁶ Wien. klin. Wchn., 1916, 29, 249.

⁷ Presse méd., 1919, 27, Annex, 629.

⁸ Riforma Med., 1920, 36, 350.

⁹ Wien. klin. Wchn., 1919, 32, 840.

¹⁰ Jour. Amer. Med. Assoc., 1916, 66, 1758.

¹¹ Glasgow Med. Jour., 1918, xc, 280.

¹² Wien. klin. Wchn., 1917, 30, 805.

¹³ Dermat. Wchn., 1918, lxvi, 384.

¹⁴ Münch. med. Wchn., 1919, lxvi, 1441.

treatment of subacute and chronic gonorrheal complications; much less so, however, in acute gonorrhea. The beneficial effects are apparently due to a combination of agencies, but it is significant that those who have employed this therapy have found the febrile reaction necessary for beneficial effects. It is possible that fever alone is destructive for gonococci; it is well known that the organisms are very susceptible to temperatures above 98° to 99° F. Due care and caution, however, must be exercised in employing these remedial measures and especially when sera and vaccines are given intravenously.

Vaccines and Non-specific Agents in the Treatment of Buboos.—Vaccine treatment has been generally confined to the treatment of chronic discharging, suppurative adenitis; autogenous vaccines prepared of organisms secured in cultures of the pus are to be preferred. If administered subcutaneously the injections should be given at intervals of five to seven days in doses large enough to secure mild focal reactions. For this purpose the vaccine may contain 2,000,000,000 organisms per cubic centimeter and treatment begun with 0.1 c.c. and gradually increased.

Luithlen has reported favorably upon the treatment of old torpid venereal ulcers associated with buboes by intramuscular injections of vaccine in doses of 300,000,000 to 800,000,000; better results were observed from two or three intravenous injections of 50,000,000 at intervals of several days.

Trossarello has employed intramuscular injections of milk in the treatment of these cases.

Vaccines and Non-specific Agents in the Treatment of Salpingitis.—Much less has been reported on the treatment of gonococcus complications in women by vaccines and non-specific agents than in the treatment of men. Gonorrheal salpingitis and ovaritis or more general pelvic inflammation is usually less acute than gonorrheal metastases in men and medical assistance is less frequently sought.

Ordinary gonococcus vaccine administered by subcutaneous injection in usual amounts has proved valueless in the treatment of subacute and chronic pelvic infections of women.

Intravenous injections of 50,000,000 to 100,000,000 gonococci, producing a general reaction similar to that previously described and usually associated with increased pelvic pain and tenderness, has yielded much better results. Five intramuscular injections at intervals of three to four days of 5 to 10 c.c. of milk boiled for ten minutes and cooled have yielded particularly good results in the hands of Trossarello.¹ All of a series of cases of tubal and ovarian disease were improved, some after a single injection. Best results were observed in those patients developing febrile reactions.

More recently Fuchs,² Schubert,³ Zoeppritz,⁴ and Sonnenfeld⁵ have reported particularly good results in the treatment of adnexal disease by injections of turpentine. The usual dose has been 0.5 c.c. of a mixture of 1 part turpentine and 4 parts sterile olive oil by intramuscular injection (gluteal). A small amount of novocain may be added to prevent local pain. Fuchs states that cases with a temperature reaction of 1° to 2° C. gave the most striking clinical results and that in over two hundred injections there was no abscess formation or untoward effects; even in acute early adnexal inflammation accompanied by much pain and hemorrhage the injection of one to two doses is usually followed by a reduction of the adnexal swelling and a decrease of pain and hemorrhage.

¹ *Riforma med.*, 1920, 36, 350.

² *Zentralbl. f. Gynäk.*, 1920, xlii, 52.

³ *Zentralbl. f. Gynäk.*, 1919, xliii, 468.

⁴ *Zentralbl. f. Gynäk.*, 1919, xliii, 297.

⁵ *Berl. klin. Wchn.*, 1920, lvii, 707.

TREATMENT OF ACUTE AND CHRONIC ARTHRITIS

Infection and Immunity in Arthritis.—Acute and chronic arthritis, including rheumatoid arthritis or arthritis deformans, are believed to be microbic infections; metabolic changes are considered secondary rather than primary etiologic factors. Gout is an exception; the irritants deposited in the joints are metabolic products capable of exciting sterile inflammatory changes, but even in this disease the probability of bacterial infection cannot be excluded in all cases and particularly since the chemical injury may favor the localization and activity of bacteria. Closed traumatic arthritis is also an exception, but aside from these the majority of arthritides are to be regarded as microbe infections, and biologic therapy is of importance in relation to both prophylaxis and treatment.

The anatomic structure of the joints and synovial membranes favors bacterial embolism; for this reason it is to be expected that in blood infections (bacteremia, septicemia) the circulating microbes may lodge in these tissues and produce disease. It is highly probable that gonococcus arthritis is of this nature rather than caused by toxic substances in the blood or gonotoxins produced in some focus in the urogenital tract. Likewise tuberculosis, typhoid, streptococcic, staphylococcic, pneumococcic, and meningococcic infections of the joints are to be regarded as embolic infections. To the best of my knowledge no one has produced arthritis experimentally with injections of bacterial toxins, and a more reasonable assumption is that the arthritis is excited by the presence of the actual germs. However, we do know that some bacterial toxins show selective affinities for certain tissues, as tetanus toxin for the spinal cord, diphtheria toxin for the ganglia of the heart, etc., and it is possible, but not probable, that poisons produced by known and unknown microbes may have a similar selective affinity for the synovial membranes of joints; simply because blood-cultures fail to detect a bacteremia is not of much significance because bacteremia is usually intermittent. Sterile cultures of joint fluid are of more significance, but do not preclude the presence of bacteria in the tissues of the joint.

Whether or not races of bacteria acquire selective affinities for the tissues of the joints is still an open question; undoubtedly the work of Rosenow and others with the streptococci are of a convincing nature and especially in relation to the subject of focal infection.

That bacteria and particularly pathogenic cocci may gain access to the blood from foci of infection in the tonsils, accessory nasal sinuses, gums and apices of the teeth, chronic bronchitis, intestines and genito-urinary tracts of both sexes with secondary localization in the joints and elsewhere, may be regarded as definitely proved. The sudden exacerbation of an arthritis following the extraction of teeth or the administration of an autogenous vaccine prepared of the bacteria from apical abscesses, indicate a very direct relationship aside from the abundance of clinical and experimental data in support of this contention.

Tuberculosis, typhoid, gonococcic, staphylococcic, pneumococcic, and meningococcic infections of the joints are distinct clinical entities and almost invariably secondary to some well-known primary infection. Chronic rheumatism, rheumatic arthritis, or arthritis deformans is a more or less well-defined clinical entity, but the exact nature of the infecting organism is unknown except that it is almost certainly a coccus. Whether it is an ordinary streptococcus or a special streptococcus rheumaticus or the diplococcus rheumaticus of Poynton and Paine cannot be stated. Likewise the exact etiology of acute rheumatic arthritis is unknown, although the majority

of investigators have found cocci and the *diplococcus rheumaticus* of Poynton and Paine has received most confirmation. It is highly probable that both the acute and chronic forms of arthritis are due to the same coccus or cocci, belonging to the group of streptococci and pneumococci.

Acquired immunity in arthritis infections never reaches a high degree. One attack of rheumatic arthritis never confers a lasting immunity against subsequent attacks; indeed, a hypersusceptibility to recurrent attacks is usually apparent probably due more to the results of injury to the joints than a decrease of natural defenses. Age, sex, seasons, exposure, and trauma are modifying factors, but with the exception of trauma, exert their effects more by exercising a modifying influence upon the primary foci producing the organisms than upon the joints. Immunity to the pathogenic cocci is never developed to a high degree; this is particularly true of gonococci and streptococci. One attack of gonorrhea and even of gonococcus arthritis does not confer any immunity against subsequent attacks; indeed, the involved joints are more susceptible than ever even though apparently healed. The same is true of the streptococci which probably include the exciting cause of acute rheumatic fever and chronic rheumatic arthritis. Doubtless some degree of local and general immunity are acquired, and particularly the development of opsonins, but these defenses are apparently feeble against fresh accessions of the organisms from the feeding places of primary focal infection. For this reason detection and removal of the primary focus is the first principle of treatment. In acute rheumatic fever, however, a primary focus of infection may not exist; indeed, it would appear that the germ may enter through the upper respiratory tract and particularly the tonsils, and induce the disease as a specific infection without producing a way station of infection.

Resistance and recovery from arthritis is probably largely brought about by the destruction of the microbes by leukocytes aided by high temperature and other non-specific agencies.

Serum Treatment of Arthritis.—The serum treatment of gonococcus arthritis has been previously described. In the acute embolic arthritis due to streptococci, meningococci, and pneumococci developing in the course of general infections by these organisms, serum treatment if demanded consists of the intravenous injection of large doses of the corresponding immune serum. Moore¹ has found that the intravenous injection of polyvalent antistreptococcus serum in rabbits prevents the development of arthritis and other lesions following the injection of streptococci capable of producing arthritis in untreated controls. Nicoll² found that antistreptococcus serum was of little or no value in chronic arthritis; however, some benefit from non-specific reactions would be expected.

Some cases of gonococcus arthritis have been treated with injections of serum directly into the involved joints; I do not know of cases of other infections treated in this manner, although the local treatment of arthritis by the injection of chemotherapeutic agents and sera directly into the joints will probably receive an increasing amount of attention in the future.

Further investigations in the etiology of acute rheumatic fever and the preparation of an immune serum for treatment are urgently required; the disease would appear to be one demanding a therapy of this kind in view of its sudden onset, severe symptoms, and progressive involvement of different joints.

Treatment of acute and chronic arthritis by *normal serum* will be considered under Non-specific Therapy.

¹ Jour. Infect. Dis., 1914, 15, 215.

² Jour. Amer. Med. Assoc., 1914, 63, 2225.

Cases of acute and chronic arthritis with effusion have also been treated—and apparently with some degree of success—by aspirating the joint and immediately injecting 1 to 5 c.c. of the fluid subcutaneously or intramuscularly (*auloserum therapy of arthritis*). When the fluid is clear, heating before injection is not required; if, however, there is reason to believe that bacteria are present, the fluid should first be heated at 60° C. for one hour. The fluid should be aspirated into sterile citrate solution (1 c.c. of 2 per cent. for 10 c.c. of fluid) to prevent coagulation. The effects are probably non-specific and probably could be improved by injecting the fluid intravenously.

Vaccine Treatment of Arthritis by Subcutaneous Injection.—The vaccine treatment of gonococcus and typhoid arthritis has been previously described.

Lacking the specific organism of *acute rheumatic fever*, vaccines administered by subcutaneous injection have not been commonly employed; this disease, however, has been successfully treated by intravenous injections of typhoid vaccine and other non-specific agents described below.

Chronic rheumatic arthritis and *arthritis deformans* have been extensively treated with vaccines prepared by streptococci, pneumococci, staphylococci, and other bacteria secured in cultures of the tonsils, apices of teeth, accessory sinuses, and other places of suspected or known foci of infection. Vaccines have also been prepared of streptococci secured in cultures by Rosenow's methods from excised lymph glands draining the involved joints, from contracted and diseased adjacent muscles, and from excised portions of the diseased capsule of the joint itself. These vaccines have been injected subcutaneously at intervals of five to seven days in amounts varying from 10,000,000 to 2,000,000,000 of cocci.

The results have not been encouraging. The most to be expected in the treatment of chronic arthritis would be the gradual relief of subjective symptoms and particularly pain and a decrease in the number and severity of acute exacerbations of the disease. Restoration of function and disappearance of deformities cannot be expected except to a slight degree and is entirely dependent upon the amount of absorption of new fibrous and calcareous tissue that may take place. In some instances these objects are apparently attained, but in well controlled studies it is evident that the same beneficial effects may be secured by the usual hygienic treatment.

To secure any benefit at all it is necessary to inject amounts of vaccine capable of eliciting a mild febrile reaction and some focal reactions in the involved joints. For this purpose 500,000,000 to 2,000,000,000 cocci per cubic centimeter may be required, depending upon the activity of the disease, and preferably by intramuscular injection. Not infrequently these injections are followed by considerable symptomatic relief and general improvement of the patient for temporary periods.

From a theoretic standpoint continuous vaccine treatment is indicated and especially if focal reactions occur after small doses, indicating that the vaccine very probably contains the causative organism. Antibody production is slow, as previously discussed, but apt to be continuous, with the probability of reaching a point when some therapeutic usefulness may become apparent even if nothing more than to delay or prevent the progression of the disease with the involvement of new joints.

Likewise when arthritis is early and involving a few of the smaller joints, but develops acute exacerbation following the extraction of teeth for apical abscesses, I believe it is good practice to secure the streptococci from such foci and institute a course of vaccine treatment for its possible prophylactic value against progression of the arthritic infection. For this purpose I

prepare an autogenous vaccine containing 3,000,000,000 cocci per cubic centimeter and start treatment with the subcutaneous injection of 0.1 c.c. Subsequent doses are given at intervals of five to seven days in gradually increasing amounts until as much as 1 c.c. is being given at one time; a series of twelve to fifteen doses are given and the balance of the vaccine carefully preserved in a cold place for future use if necessary.

Non-specific Treatment of Arthritis with Special Reference to the Intravenous Injection of Typhoid Vaccine.—Miller and Lusk¹ were the first to report on 24 cases of arthritis treated with intravenous injections of proteoses and typhoid vaccine. A second series of 85 cases were given intravenous injections of 40,000,000 to 75,000,000 typhoid vaccine. Forty-five of these were cases of acute arthritis, and 33 of the patients had already been treated with salicylates, without benefit, except in 4 cases. Twenty-nine out of the 45, after receiving one to four doses of vaccine, recovered in one to five days; 8 showed marked improvement. The 4 gonococcus cases in this series did not respond well to the vaccine. There were 9 relapses in the acute series. Of 12 cases of subacute arthritis, 10 cleared up in three to five days, and the other 2 patients improved. Nineteen chronic, but still active cases were treated, in 10 of which the patients showed improvement. Culver² reports a series of 31 gonococcus arthritis cases, in 28 of which the patients either recovered or greatly improved after intravenous injection of gonococcus, meningococcus, or colon vaccine. Manier, Petersen, and Jobling³ have treated 13 patients with arthritis by intravenous injections of secondary proteoses. Three of the cases were acute, 3 subacute, and 7 chronic. Of the acute cases, 2 cleared up promptly after the injections, while in the third, a gonococcus arthritis, the patient was not relieved. In the 3 subacute cases the patients all recovered rapidly after treatment. In the chronic series there was complete relief in 3, marked improvement in 3, and no change in 1. Cecil⁴ has treated 40 cases of arthritis, of which 26 were of the ordinary rheumatic type, 7 acute toxic arthritis, and 7 of gonorrheal origin. Of the rheumatic and toxic arthritides, 40 per cent. recovered in from two to ten days without the use of salicylates. Of 20 patients receiving salicylates in addition to the vaccine, 17 were cured or greatly benefited. The 7 patients with gonococcus arthritis made very slow improvement. Cecil noted that while the arthritic pains were generally relieved, muscular pains persisted in many cases.

Snyder,⁵ Thomas,⁶ Scully,⁷ Pemberton,⁸ Cross,⁹ Cadbury,¹⁰ Cowie and Calhoun,¹¹ Boyd,¹² Gow,¹³ and others have employed intravenous injections of typhoid and other vaccines with favorable results especially in the acute and subacute cases; Hardinger¹⁴ reports that his results were not satisfactory. In Europe intramuscular injections of milk and casein and intravenous injections of casein have been more commonly employed, and a few investi-

¹ Jour. Amer. Med. Assoc., 1916, 66, 1756; *ibid.*, 1916, 67, 2010.

² Arch. Int. Med., 1917, 19, 1042.

³ Jour. Amer. Med. Assoc., 1916, 66, 1753; Arch. Int. Med., 1917, 19, 1042.

⁴ Jour. Amer. Med. Assoc., 1917, 69, 20.

⁵ Arch. Int. Med., 1918, 22, 224.

⁶ Jour. Amer. Med. Assoc., 1917, 69, 770.

⁷ Jour. Amer. Med. Assoc., 1917, 69, 20.

⁸ Arch. Int. Med., 1920, 25, 351.

⁹ Jour.-Lancet, 1917, 37, 764.

¹⁰ China Med. Jour., 1919, 33, 213.

¹¹ Arch. Int. Med., 1919, 23, 69.

¹² Jour. Lab. and Clin. Med., 1919, 5, 88.

¹³ Brit. Med. Jour., 1920, 1, 284; Quart. Jour. Med., 1919, 13, 82.

¹⁴ Colorado State Jour. Med., 1921, 19, 26.

gators have used intravenous injections of proteoses and peptone; extracts of cartilage, the "sanarthrit" of Heilner,¹ have been employed abroad and especially in the treatment of gout by Umber,² Sterna,³ and others.

Dosage and Administration.—Ordinary typhoid vaccine has been most commonly employed for eliciting the non-specific reaction in arthritis. The amount injected intravenously has varied from 10,000,000 to 100,000,000; larger amounts, up to 500,000,000, have been injected in a few instances without greatly increasing the reaction and with no ill effects, but the smaller amounts are sufficient and safer.

For beneficial effects a sharp reaction must be produced, and the degree of reaction varies in different individuals receiving the same substance in the same amount.

Intravenous injections have yielded the best results. As previously stated, typhoid vaccine has been mostly employed, but colon vaccine and albumose may be employed. For the average adult case the amounts injected for the first dose may be as follows; the subsequent injections should be larger or smaller depending upon the degree of reaction and results:

(a) Ordinary typhoid vaccine in dose of 50,000,000. The typhoid-paratyphoid vaccine used for prophylactic immunization is satisfactory and the stock vaccine containing a total of 500,000,000 bacilli per cubic centimeter may be employed by taking 1 part and diluting with 9 parts of sterile saline solution and injecting 1 c.c. intravenously. If the vaccine contains 1,000,000,000 bacilli per cubic centimeter, 1 part should be diluted with 19 parts of saline and 1 c.c. injected to give a dose of 50,000,000.

(b) Colon bacillus vaccine in dose of 25,000,000 intravenously.

(c) A 5 per cent. solution of Merck's albumose in sterile saline solution sterilized by boiling for ten minutes; dose 1 c.c. diluted with 4 c.c. of sterile saline intravenously.

(d) Ordinary market milk boiled for ten minutes, cooled, and injected intramuscularly in dose of 10 c.c. Usually one or two intramuscular injections are sufficient; if beneficial effects are not observed after the first, providing a reaction has been produced, it is entirely likely that further injections will not bring about an improvement.

Injections should not be given until the effects of the previous one has subsided. The interval is generally five or more days. The average case requires one to three injections, but as many as ten have been given.

The Reactions.—These have been described in detail on p. 881. As previously stated, a general and focal reaction must be produced in order to elicit a beneficial effect upon the joints. Delirium may occur among alcoholics and marked dyspnea has developed in a few cases. There can be no doubt that the reaction is unpleasant and may be dangerous when the injections are given to improperly selected patients. The chill sometimes is unusually severe and results in rendering the joints more painful than ever by reason of the involuntary movements of muscles and joints. As a general rule, however, the after-effects and particularly relief from pain are so marked that many patients request subsequent injections, and if alcoholics and patients with acute endocarditis and advanced myocardial degeneration and hypertension are excluded, there is very little risk.

Contraindications.—Since this treatment may excite delirium tremens, alcoholics should be selected and treated with extra caution or excluded altogether; cases of arthritis with acute endocarditis and chronic cases with

¹ Münch. med. Wchn., 1916, 63, 997; *ibid.*, 1918, 65, 983.

² Münch. med. Wchn., 1918, 65, 988.

³ Münch. med. Wchn., 1920, 67, 632.

well-marked myocardial degeneration are also to be considered as risks for intravenous medication. Intramuscular injections of milk are less dangerous and may be employed in these cases. Young vigorous individuals are best adapted for intravenous therapy.

Results and Practical Value.—Non-specific therapy probably has no influence upon the primary foci of infection, and a diligent search should be made for these and adequate treatment applied. It is probable, however, that the leukocytosis, fever, ferment, and antibody increase elicited by the sharp protein reaction may have a direct destructive effect upon the microbes in the joints, so that further progression of the arthritis may be prevented, but direct proof of these facts is lacking. "Whether as a result of the reaction the local tissues become immune to the toxic effect of bacteria still alive in the focus, whether it means merely an increased tolerance to toxic split products set free at a distance and to which the local tissues had heretofore been sensitive, or whether we deal with the actual destruction of bacteria which had become localized in the joint is not determined" (Petersen).

Cowie¹ has recently summarized the results very well as follows:

"It cannot be questioned that very remarkable beneficial effects have followed the intravenous injection of foreign protein in the arthritides. There is seldom a case of acute arthritis or peri-arthritis which does not respond to a certain degree, but this improvement is often of only a few hours' duration. Taking cases at random, including all varieties, there will be found a large percentage in which no permanent beneficial effect can be secured by this method of treatment. One should not expect permanent beneficial effects in cases of peri-arthritis of a less chronic nature, unless great care has been taken to rid the body of all foci of infection that can possibly be found. Accordingly, therapeutic intravenous injections of foreign protein should follow failure to secure successful results by the removal of such foci of infection, or they should be used in conjunction with an attempt to remove the focus.

"Unquestioned relief from pain will often follow protein therapy, even though the focus of infection is not removed, and, in some cases, in addition to improvement in the joint condition, the focus itself may cease to be active."

"Thus far, experience has taught us that acute or subacute processes that have not progressed beyond the first year are the ones that give the best results, particularly those that have not gone on to marked structural change of the articular or periarticular tissue; next to these, cases which have progressed longer and which may show structural change, but which have not produced definite ankylosis and its consequential results."

I believe that the work that has been done justifies the statements that acute and subacute arthritis and peri-arthritis are the forms which respond more promptly and more surely to this method of treatment. A few cases of chronic arthritis of as long as three years' standing have been recorded in which apparently complete cure has followed this method of treatment; and in still more chronic forms, unquestioned benefit has occasionally resulted. However, at present there are not enough properly classified cases recorded to enable us to say what percentage of each class is benefited by this method of treatment.

Petersen states that "in perhaps 40 per cent. of the cases one or two injections completely terminate the disease, in another 30 per cent. the improvement is marked and recovery made complete on further injections,

¹ Jour. Amer. Med. Assoc., 1921, 76, 310.

while in the balance there may be either a transient improvement with a relapse later, or no marked clinical improvement."

Acute and subacute cases of non-gonorrheal origin have yielded the best results. The most to be hoped for in chronic arthritis is relief of the joint pains—muscular pains may not be relieved—and a cessation or retardation of further progress of the disease. Restoration of function is not to be expected unless gradually brought about by passive movements. The absorption of inflammatory deposits and release of muscular fibrosis and contraction does not take place, although miracles of this sort are commonly expected to occur when this and other forms of biologic therapy are employed.

Non-specific therapy has no curative effect upon cardiac disease; both Torrey and Petersen believe, however, that under this treatment serious cardiac involvement is less likely to occur.

TREATMENT OF DISEASES OF THE SKIN

The Skin in Relation to Infection, Immunity, and Biologic Therapy.—

In a broad and general manner diseases of the skin may be divisible into those produced by local microparasites and those developing during the course of general infections and pathologic alterations of internal organs. As discussed in Chapter IX, the skin is not only an important barrier to infection but is probably concerned in an intimate manner in the mechanism of recovery from infectious and non-infectious diseases, and by reason of these relationships is subjected to many different kinds of injury.

The treatment of skin diseases, therefore, is a broad and general field. As Ravaut is quoted: "To treat skin disease wholly from without is as irrational as treating the skin lesions of syphilis by local applications alone."

Furthermore, it would appear that the skin is an organ capable of producing ferments, antibodies, and possibly internal secretions, profoundly influencing the metabolism and diseases of the internal organs. By reason of these activities it is very probably concerned in the processes of recovery from many diseases not directly involving the skin; furthermore, it would appear that these remedial agencies may be brought into play by various therapeutic agencies. For example, the curative effects of light rays and sunlight upon different diseases of the skin and of the internal organs as well, notably tuberculosis, may be due to mild non-specific shock instead of the production of some hypothetic internal secretion stimulating the organism. As stated by Petersen¹: "The epithelial tissues become hyperemic and absorption from them is accelerated. Skin enzymes—protease and lipase, perhaps some ereptase in younger individuals—are swept into the circulation, together with some protein-split products due to digestive stimulation in the skin. The agents that ordinarily provoke the non-specific reaction are, therefore, available; the enzymes present in the serum can now attack seminecrotic or necrotic foci and there set up a focal reaction with its resulting train of increased temperature, malaise, etc." Hoffmann² has also called attention to the fact that other therapeutic measures, as counterirritation, blistering, etc., may involve precisely the same mechanism.

The work of Müller³ also affords a striking example of the importance of skin stimulation on remote disease processes. He has shown that the amount of antigen required to produce a focal reaction about a disease process remotely situated, *i. e.*, gonorrheal urethritis, may be one-thirtieth less

¹ Protein Therapy and Non-specific Resistance, MacMillan Co., 1922, 138.

² Strahlentherapie, 1919, 7, 1.

³ Münch. med. Wchn., 1920, lxxvii, 9; Med. Klinik, 1920, 26, 579.

when injected intracutaneously than when injected intramuscularly or intravenously. These observations indicate that slight skin reactions may exert an important effect on remote pathologic lesions irrespective of the substance injected and to be ascribed to changes and processes produced in the skin. On the basis of these observations Ponndorf¹ and Kroschinski² have employed intracutaneous injections of tuberculin not only for the treatment of tuberculosis but in neuralgia, neuritis, acne, furunculosis, and other diseases.

Treatment of Acne.—Acne vulgaris was among the first diseases in which vaccine therapy has been applied. The cause of the disease is not definitely known and may have some relationship to overstimulation of the sebaceous glands by disturbances of glands of internal secretion and especially those concerned with sexual changes at puberty and early adult life. Schamberg also regards intestinal auto-intoxication (predisposed to by constipation) as a factor of importance; in many cases the foodstuffs favoring the development of toxic substances are the starches and sugars. Of interest in this connection are the results of complement-fixation tests with the sera of individuals suffering with acne conducted by Strickler, Schamberg, and the writer³; a large percentage were found to yield positive reactions with antigens of colon bacilli, indicating that this group of organisms may bear a relationship to the disease in at least some cases.

It would appear that while metabolic changes of this kind are of etiologic significance, doubtless bacterial activity in the skin is also of importance. The *acne bacillus* described by Unna, Sabouraud, and Gilchrist is especially found in myriads, in comedones, and in sebaceous glands. Staphylococci are invariably found in the pustular lesions. It is held by some that in acne there is a follicular hyperkeratosis which obstructs the hair follicles and sebaceous glands and results in retention of secretion, inflammation, and suppuration.

It is apparent, therefore, that *vaccine therapy* is only a part of the treatment of acne. The results of vaccine treatment are sometimes brilliant, at other times very disappointing, but probably in the majority of cases of distinct aid.

Engman⁴ has observed particularly good results in the treatment of acne indurata and certain forms of cystic acne, with stock vaccines of the *acne bacillus*. Less favorable results were observed in the more superficial types, known as acne simplex and acne pustulosa. Engman has given the following directions as to dosage: "The initial dose should be from 3,000,000 to 5,000,000, to be repeated in from five to seven days. The interval should be gaged according to the reaction to the dose, which is usually exhibited by the appearance of a few new lesions within forty-eight hours after the injection. On the third day after the injection comedones may be expressed and the lesions opened if necessary. The manipulation of the lesions at this time helps bring the immunizing blood to the part and it is at the height of the "tidal wave of immunity." Local hyperemia in the form of hot towels or that resultant from manipulation should be used on the third day after each injection and not before then.

If, after a few such doses, new lesions continue to appear after the third day, a larger dose of from 7,000,000 to 10,000,000 should be given and continued until a proper therapeutic result is obtained, when the interval of

¹ Münch. med. Wchn., 1914, lxi, Nos. 14 and 15.

² Münch. med. Wchn., 1921, lxxviii, 205.

³ Jour. Cutan. Dis., March, 1916.

⁴ Jour. Amer. Med. Assoc., 1921, 76, 176.

administration should be lengthened to two weeks, then three weeks, and finally four weeks, thus continuing the remedy in a prophylactic manner.

If there is an outcrop of many new lesions within forty-eight hours after injection the dose should be lessened and the interval of dosage extended. However, if many new lesions appear after the third day it is an indication for a much larger dose. In no instance should more than 15,000,000 be administered in any injection."

Engman states that staphylococcus vaccine or a mixed vaccine of staphylococci and the acne bacillus are of little value, except in some cases of acne varioliformis, which occurs later in life.

In my experience best results have been observed when a mixed vaccine of the acne bacillus, staphylococci, and the colon bacillus has been employed, and especially in the treatment of the pustular infections. Since the acne bacillus is a difficult organism to cultivate a stock suspension is employed; the staphylococci, however, are secured in cultures of the lesions and the colon bacillus secured by plate cultures of the feces. The mixed vaccine is so prepared that each cubic centimeter contains 200,000,000 acne bacillus and 400,000,000 each of the colon bacillus and staphylococci. The first dose is 0.1 c.c.; subsequent injections are given at intervals of five to seven days in gradually increasing amounts according to the plan of treatment employed by Engman for the administration of vaccines of the acne bacillus.

Treatment with non-specific agents has been employed by numerous investigators. Intravenous injections of 50,000,000 *typhoid bacillus vaccine* has brought about improvement and may be considered in the treatment of severe cases. *Turpentine* injections have been employed by a number of physicians and especially Klingmueller,¹ who injects about 4 drops of a 20 per cent. solution in sterile olive oil subcutaneously every three or four days over a long period of time. Intramuscular injections of 5 to 10 c.c. of *milk*, boiled and cooled, have been employed and apparently with success in some cases.

Treatment of other Pyogenic Dermatoses; Sycosis Vulgaris; Impetigo Contagiosa; Ecthyma (Pyodermia).—*Autogenous vaccines* are sometimes of value in the treatment of these pustular infections. As a general rule cultures reveal the presence of staphylococci. Vaccines of staphylococcus aureus, frequently found in cultures of the deeper lesions, are particularly apt to prove helpful in some cases, as reported by Dennie and Bufford,² McLeod,³ and others.

The vaccine may be so prepared that each cubic centimeter contains 1,000,000,000 cocci. The first dose should be 0.1 c.c. and subsequent injections given at intervals of five to seven days in gradually increasing amounts.

Subcutaneous injections of 20 per cent. dilutions of *turpentine* and intramuscular injections of *milk*, have likewise been employed with some success; the doses are the same as those given above for the treatment of acne.

Treatment of Eczema.—True eczema is very probably the cutaneous manifestations of general, functional, and metabolic disturbances in which bacterial infection may be responsible for secondary changes.

The relation of food allergy to eczema, its diagnosis by skin tests, and treatment, have been described in previous chapters.

The term "eczema" is a very broad and general one, including a variety

¹ Berl. klin. Wchn., 1918, 45, 896; Münch. med. Wchn., 1918, 55, 896.

² Boston Med. and Surg. Jour., 1916, 173, 905.

³ Practitioner, London, 1920, 104, 338.

of dermatoses, produced by a variety of widely different causes. No other skin affection requires more thorough study and search for etiologic factors than this group.

In eczema papulosum, vesiculosum and pustulosum, and in infectious eczematoid dermatitis of Fordyce originating around suppurating wounds autogenous vaccines may be of some aid in treatment as reported by Medalia¹ and others. As a general rule staphylococci and various diplococci are to be found; the vaccine may contain 1,000,000,000 per cubic centimeter. Treatment is begun with 0.1 c.c. and the injections given every five to seven days in gradually increasing amounts.

Subcutaneous injections of 20 per cent. *turpentine* in sterile olive oil in dose of 2 to 4 drops every three or four days have been employed by Klingmueller,² Singermann,³ Becker,⁴ and others. Intramuscular injections of *milk* boiled for ten minutes and cooled have also been employed with success; the dose for adults is 5 to 10 c.c. and for children correspondingly smaller amounts. *Typhoid vaccine* in dose of 50,000,000 has been injected intravenously by a few observers.

Spithoff,⁵ who has been one of the earliest advocates of the autoserum treatment of certain skin diseases, has reported favorable results from the *autoserum* treatment of eczema. He draws from 50 to 100 c.c. of blood from adults, separates the serum and reinjects the serum intravenously on alternate days at first and then twice a week. Heuck,⁶ Ullman,⁷ and others, however, have not noted any beneficial effects of autoserum injections in eczema.

Danysz's⁸ *enterovaccine* has been employed in the treatment of various non-infectious diseases of the skin, including eczema, with apparent success. He has worked on the theory that these diseases may be anaphylactic phenomena and that albuminoid material or microbial constituents in the intestinal canal may pass into the blood and act as antigens with the production of anaphylaxis. He now employs a stock antigen prepared by cultivating a scrap of feces in ordinary bouillon and then securing pure cultures, which are mixed in the same proportions as found originally. This vaccine is diluted with saline, sterilized with heat and the dose determined by weight. For oral administration, the dose is 1/10 to 5/10 mg.; for subcutaneous injection 1/1000 to 1/1200 mg.

Treatment of Psoriasis.—The etiology of psoriasis is still unknown. *Vaccines* prepared of various staphylococci, diplococci, diphtheroid bacilli, etc., secured in cultures of psoriatic lesions, have been employed in treatment by a large number of physicians. In not a few instances cures have been claimed, but the disease is so subject to periods of spontaneous improvement that the evidence in favor of the efficacy of vaccines is not convincing. In the writer's experience autogenous vaccines of the cutaneous organisms have proved of no value.

Various non-specific biologic agents, however, have been employed with some evidence of success, and particularly injections of the patient's own serum (autoserum therapy), while chrysarobin ointment (2 to 10 per cent.) is being applied locally.

¹ Boston Med. and Surg. Jour., 1915, 173, 187.

² Boston Med. and Surg. Jour., 1915, 173, 187.

³ Derm. Zentralb., 1920, 23, 130.

⁴ Derm. Wchn., 1920, lxxi, 472, 481.

⁵ Med. Klinik, 1914, 11, 29; *ibid.*, 1916, 12, 1223.

⁶ Münch. med. Wchn., 1912, lix, 2608.

⁷ Arch. f. Dermat. n. Syph., 1913-14, cxviii, 125.

⁸ Presse Méd., 1918, 26, 367; Bull. Med., 1920, 34, 155, 657.

Autoserum treatment was first employed by Spiethoff¹ for the treatment of psoriasis and apparently with considerable success. Gottheil and Satenstein,² in this country, have likewise reported favorably. Fox³ found autoserum injections in general very satisfactory and in some cases the results were decidedly brilliant, but only when used in conjunction with chrysarobin. Similar results were observed by Hilario,⁴ Ravitch,⁵ and Trimble and Rothwell⁶; Willock⁷ has not observed any beneficial effects from autoserum injections in psoriasis. Schamberg and myself have observed apparently beneficial results in some cases and especially those with acute exacerbations unable to bear with chrysarobin ointment. Not infrequently the injections resulted in rendering the lesions quiescent and when employed with applications of neorobin ointment yielded beneficial results.

The technic of these injections has been described in Chapter XXXVII. As a general rule 50 c.c. of blood is drawn from a vein at the elbow under aseptic precautions, the serum separated (usually 15 to 20 c.c. obtained) and injected intravenously. Several injections are given at intervals of about seven to ten days.

Linser⁸ and Perry⁹ have employed six to nine intravenous injections of 3 to 5 c.c. of horse-serum. Engman and McGarry¹⁰ and Scully¹¹ have employed intravenous injections of typhoid vaccine in dose of 75,000,000 to 100,000,000. The latter reports that these injections do not clear up the lesions of psoriasis, but lessen the induration and inflammatory reaction. When employed with the local application of weak chrysarobin ointment the lesions frequently cleared away with rapidity. Cadbury¹² observed temporary improvement in 5 cases, all relapsing sooner or later. Turpentine and milk injections have also been employed in doses similar to those given above in the treatment of acne.

Sabouraud¹³ considers that non-specific therapy has introduced a new era in the treatment of psoriasis and finds that improvement occurs in most cases and lasts longer under treatment with Danysz's *enterovaccine* than any other measures yet known. This vaccine has been described under Eczema.

Treatment of Ringworm (Trichophytosis).—Vaccines of the small-spored fungus *Microsporon audouini* and of the large-spored fungus *trichophyton*, have been reported by Strickler,¹⁴ Engman and McGarry,¹⁵ and others as yielding good results in the treatment of ringworm of the body (*tinea circinata*) and of the scalp (*tinea tonsurans*). The deep-seated lesions have improved under this treatment more rapidly than the superficial ones. Autolytic products of the fungi designated as *trichophyton* have been employed as vaccines by various European observers.

Strickler has prepared the vaccines by cultivating the fungi on Sabouraud's solid "French proof agar," removing the growth, grinding with

¹ Münch. med. Wchn., 1913, ix, 521.

² Med. Record, 1914, lxxxv, 620; Jour. Amer. Med. Assoc., 1914, 63, 1190.

³ Jour. Amer. Med. Assoc., 1914, 63, 2190; Jour. Cutan. Dis., 1915, 33, 616.

⁴ Jour. Cutan. Dis., 1914, 32, 780.

⁵ Jour. Amer. Med. Assoc., 1915, 64, 1228.

⁶ Jour. Cutan. Dis., 1915, 33, 621.

⁷ Jour. Amer. Med. Assoc., 1916, 65, 14.

⁸ Verhandl. d. deutsch. Kong. f. inn. Med., 1911, 28, 125.

⁹ Boston Med. and Surg. Jour., 1916, 174, 274.

¹⁰ Jour. Amer. Med. Assoc., 1916, 67, 1741.

¹¹ Jour. Amer. Med. Assoc., 1917, 69, 1684.

¹² China Med. Jour., 1919, 33, 213.

¹³ Presse méd., 1920, 28, 53.

¹⁴ Jour. Amer. Med. Assoc., 1915, 65, 224.

¹⁵ Jour. Amer. Med. Assoc., 1917, 68, 543.

weighed amounts of crystals of sodium chlorid, and diluting with sufficient sterile water to give a fairly turbid emulsion in isotonic saline solution. This suspension was heated to 60° C. for one hour, cultured for sterility, and preserved with 0.25 per cent. phenol. The dose was 1 to 4 c.c. by subcutaneous injection every three to six days.

Lavinder¹ prepares the vaccine by cultivating the fungi in flasks of about 100 to 150 c.c. of Sabouraud's liquid medium. The growth is removed by filtration through sterile paper, washed once or twice with saline, transferred to a dish or foil, and dried in the incubator. The dried material is now weighed and each gram ground with one gram of salt and gradually rubbed with 100 c.c. of sterile water. Each cubic centimeter, therefore, contains 10 mg. in isotonic saline. The emulsion is heated to 60° C. for one hour, preserved with 0.25 per cent. phenol or tricrosol, and cultured for sterility. The first dose is 0.3 or 0.4 c.c. (3 or 4 mg.) and subsequent doses are gradually increased.

Fischl,² Müller,³ Grabisch,⁴ and others have reported good results in the treatment of trichophyton infections with subcutaneous injections of 20 per cent. solutions of turpentine in sterile olive oil; the initial dose is about 0.2 c.c. and subsequent doses at intervals of three to five days are gradually increased. Especially good results were observed in deep lesions. Löwenfeld and Paulay⁵ observed as good results in the treatment of these infections with such non-specific agents as tuberculin and turpentine as with specific vaccines ("trichon"). Ruete,⁶ however, did not observe beneficial effects from injections of turpentine.

Reese,⁷ Scholz and Kraus,⁸ and others have treated large series of cases with intramuscular injections of 5 to 10 c.c. of boiled milk and regard this type of non-specific treatment as yielding better results than turpentine injections.

It is apparent, therefore, that in severe cases of ringworm of the body, scalp, and bearded region treatment by subcutaneous injections of vaccine, turpentine, or milk (intramuscularly) may prove valuable adjuvants to local therapy; the beneficial effects ascribed to the vaccine are probably in large part non-specific.

Treatment of Pemphigus; Urticaria; Dermatitis Herpetiformis; Prurigo; Pruritus; Lichen Planus; Strophulus and other Dermatoses; Pellagra.—Many observers have reported favorably upon the treatment of various dermatoses, notably the various forms of urticaria and pruritus with intravenous injections of the patient's own serum. Urticaria is frequently a manifestation of allergy to foods or other agents and the subject has been previously discussed from this standpoint.

In the *herpes and pruritus of pregnancy* and *vomiting of pregnancy*, Mayer and Linser,⁹ Mayer,¹⁰ Freund,¹¹ Linser,¹² Fetzer,¹³ Viel,¹⁴ Rubsamen,¹⁵ and others have reported marked relief following the intravenous injection of the patient's own serum in doses varying from 10 to 50 c.c. In senile *pruritus* marked relief is sometimes afforded by the intramuscular injection of 5 to 10 c.c. of milk, boiled for ten minutes and cooled. A few hours later a mild fever develops with local soreness, but generally with total

¹ Jour. Amer. Med. Assoc., 1916, 66, 945.

² Wien. klin. Wchn., 1919, 2, 2.

³ Münch. med. Wchn., 1918, lxxv, 697.

⁴ Dermat. Wchn., 1918, lxxvii, 624.

⁵ Wien. klin. Wchn., 1919, 32, 498.

⁶ Dermat. Ztschr., 1919, 28, 28.

⁷ Münch. med. Wchn., 1919, lxxvi, 747.

⁸ Dermat. Wchn., 1918, lxxvii, 857.

⁹ Münch. med. Wchn., 1910, lvii, 2757.

¹⁰ Münch. med. Wchn., 1911, 35, 1299.

¹¹ Med. Klinik, 1911, 7, 371.

¹² Dermat. Ztschr., 1911, 17, 217.

¹³ Deut. Gesellsch. f. Gyn., 1911, 14, 712.

¹⁴ Münch. med. Wchn., 1912, lxx, 1911.

¹⁵ Deutsch. med. Wchn., 1913, 39, 931.

relief of the intolerable itching of body and scalp which lasts for several days and even weeks, when a second injection is required.

In *urticaria*, *dermatitis herpetiformis*, *prurigo*, *pruritus*, *lichen planus*, *strophulus*, and hemorrhagic diseases of the skin and mucous membranes good results from this therapy have been reported by Linser,¹ Heuck,² von Zumbusch,³ Ullman,⁴ Spiethoff,⁵ Praetorius,⁶ Holobut and Lenartowicz,⁷ Gottheil and Sattenstein,⁸ Willock,⁹ Swann,¹⁰ and others. In many cases relief was afforded by one to three injections of the patient's own serum in dose of 10 to 25 c.c. or more, but not infrequently the beneficial effects are only temporary. The treatment would appear to be especially worthy of trial in the toxic dermatoses of pregnancy, severe urticaria, dermatitis herpetiformis, and pemphigus; cures are not commonly effected and this is especially true of pemphigus.

Palmer and Secor¹¹ have reported favorable results in the treatment of *pellagra* by producing blisters with cantharides, drawing off the serum and injecting 1 c.c. at once subcutaneously; these injections were made about once a week.

The technic of securing the blood and serum and for giving the injections, has been described in Chapter XXXVII.

Subcutaneous injections of 20 per cent. turpentine in sterile olive oil in ascending doses beginning with 0.1 or 0.2 c.c. and intramuscular injections of 50,000,000 to 75,000,000 of typhoid bacilli have also been employed in the treatment of these skin affections as well as in the treatment of *lupus erythematosus* and *lupus vulgaris*, *exfoliative dermatitis*, *purpuras* (especially with injections of milk owing to its styptic qualities), *leg ulcers*, *erythema multiforme*, *erythema nodosum*, and *actinomycosis*.

Vaccine Treatment of Pruritus Ani, Vulvæ and Scroti.—Murray¹² has reported a series of cases of pruritus ani relieved or apparently cured by the administration of autogenous vaccines of *Streptococcus fecalis* secured in cultures of the effected skin as follows: After preliminary cleansing with soap and water, flushing with sterile water, and drying with gauze, the deeper layers of the skin are exposed with a sterile skin curet, avoiding bleeding as much as possible, and cultures made on blood or ascites agar. The vaccine is sterilized with 0.5 per cent. phenol or 0.3 per cent. tricresol, and not with heat.

Injections are given subcutaneously once a week in ascending doses. With a vaccine containing 1,000,000,000 per cubic centimeter the first dose may be 0.1 c.c.

Murray states that of 181 cases distinct relief was obtained in 113, including some with severe pruritus involving the neighboring parts. Frick¹³ has also reported favorably upon the treatment of 40 cases of pruritus ani, and it would appear that autogenous vaccine therapy is worthy of trial in conjunction with appropriate local treatment.

The intramuscular injection of 5 or 10 c.c. of milk, boiled for ten minutes and cooled, has, in my experience, afforded complete relief to several patients for variable periods of time.

¹ Arch. f. Dermat. u. Syph., 1912, cxiii, 701.

² Münch. med. Wchn., 1912, lix, 2608.

³ Wien. klin. Wchn., 1913, 38, 2348.

⁴ Arch. f. Dermat. u. Syph., 1913-14, cxviii, 125.

⁵ Münch. med. Wchn., 1913, lx, 521; Med. Klinik, 1914, 11, 29; *ibid.*, 1916, 12, 1223.

⁶ Münch. med. Wchn., 1913, lx, 867.

⁷ Dermat. Wchn., 1914, lviii, 41.

⁸ Jour. Amer. Med. Assoc., 1914, 63, 1190.

⁹ Jour. Amer. Med. Assoc., 1916, 65, 14.

¹⁰ Jour. Amer. Med. Assoc., 1915, 64, 737.

¹¹ Jour. Amer. Med. Assoc., 1915, 64, 1566.

¹² Jour. Amer. Med. Assoc., 1918, 71, 1449.

¹³ Ohio State Med. Jour., October, 1919.

Vaccine Treatment of Actinomycosis.—Wynn,¹ Whittier,² Kinnicutt and Mixer,³ Collic,⁴ Malcolm,⁵ and Dean⁶ have reported the successful treatment of actinomycosis with vaccines when iodides have failed. These vaccines have been prepared of the ray fungus cultivated on Sabouraud's or other suitable media, broken up by grinding, suspended in saline solution, and sterilized by heating. The methods described above for the preparation of ringworm vaccine may be employed.

Standardization of the vaccine is rather difficult. Dean states that no more than 10,000,000 actino fragments should be given in one dose. My practice is to prepare a suspension of a density equalling tubes No. 5 to 8 of the McFarland nephelometer and begin treatment with 0.1 c.c. Subsequent injections are given at intervals of one week in ascending doses.

Treatment of Other Skin Diseases.—The treatment of *dermatitis venenata* has been discussed in Chapter XXXII; *furunculosis* has been considered in this chapter and *tuberculosis* of the skin will be considered in the following chapter on Tuberculosis Therapy.

TREATMENT OF GLANDERS

Immunity in Glanders.—This disease is caused by *Bacillus mallei* and occurs most frequently among horses and mules. Man is occasionally infected and especially stablemen. Cattle and rats possess a high degree of natural resistance; sheep, goats, dogs, and rabbits likewise possess some natural immunity but may be infected experimentally, while guinea-pigs and animals of the cat family are highly susceptible. In man the disease is usually chronic, has a mortality of about 50 per cent., and, according to Fitch,⁷ is frequently mistaken for pyemia, rheumatism, syphilis, or other disease.

The nature of the natural immunity exhibited against *B. mallei* is not known; very probably phagocytosis is an important means of resistance to infection.

During the disease in man and the lower animals various antibodies are to be found in the blood, notably agglutinins and complement-fixing antibodies. These are very useful for serologic diagnosis, as discussed in previous chapters. Allergic sensitization to *B. mallei* proteins also occurs and a product of the bacillus, called *mallein*, prepared in the same manner as old tuberculin, is widely employed by veterinarians for diagnostic purposes.

These antibodies can be produced artificially by the immunization of horses and other animals with dead and living bacilli, but vaccines are only of doubtful value for prophylactic immunization and the immune serum is of little benefit in treatment.

Immunity to glanders in the horse and other lower animals is poorly developed. In this respect the disease resembles tuberculosis. Chronic lesions may become acute and one attack does not confer immunity to subsequent attacks. This is particularly perplexing and paradoxical in view of the readiness with which antibodies are produced during the disease or by artificial immunization.

Vaccine Prophylaxis and Treatment.—The use of vaccines for the prophylaxis of glanders among horses is sometimes employed; according to some investigations a temporary immunity may be secured, but corroboration is lacking.

¹ Brit. Med. Jour., 1908, 1, 554.

² Jour. Amer. Med. Assoc., 1909, 53, 1453.

³ Boston Med. and Surg. Jour., 1912, 67, No. 3.

⁴ Brit. Med. Jour., 1913, 1, 991.

⁵ Brit. Med. Jour., 1916, 2, 488.

⁶ Brit. Med. Jour., 1917, 82.

⁷ Cornell Veterinarian, July, 1914.

Vaccines and mallein have also been employed in the treatment of glanders of horses and of human beings. The dose for human beings may be 0.1 c.c. of a vaccine containing 1,000,000,000 per cubic centimeter; injections are subcutaneous and repeated at intervals of five to seven days in increasing amounts. Bristow and White,¹ Cramp,² Zieler,³ and others have reported success with autogenous vaccines; mallein has been employed by Robins⁴ in a manner analogous to the subcutaneous injection of old tuberculin. While the disease is fortunately rare in human beings it is nevertheless important, by reason of the high mortality, and vaccine treatment should be employed along with the usual medicinal and surgical measures.

TREATMENT OF LEPROSY

Immunity in Leprosy.—Leprosy is caused by *Bacillus lepræ*, but it is still doubtful whether this organism has been successfully cultivated outside of the tissues. The disease is one of great antiquity and all races of human beings are susceptible to infection. Children are probably more susceptible than adults; whether or not the disease is transmitted in the uterus is still undecided, but probably is not.

The ordinary domestic animals enjoy an absolute immunity to the disease; rat leprosy occurs, but whether it is caused by the human bacillus is unknown, but probably is a separate infection. Monkeys have been successfully inoculated according to some investigations, but these observations require corroboration.

Man is probably infected through the upper respiratory passages and the bacilli are constantly found on the nasal mucosa of lepers. Prolonged and intimate contact is usually required for infection, and many individuals apparently escape altogether in spite of frequent and intimate exposure. Either the bacilli are but feebly pathogenic and largely destroyed by phagocytic cells (mononuclear leukocytes) or else a prolonged period of incubation is required before lesions develop—a matter of years. There is evidence indicating that bacteremia occurs and that the bacilli may be transmitted to the skin and other organs and tissues by way of the blood-stream.

Antibodies are apparently produced during the course of disease, at least to a degree sufficient for bringing about long periods of latency, spontaneous improvement, and even apparent cure in some cases. The degree of immunity, however, is usually insufficient for bringing about a cure and in the majority of cases the disease progresses to a fatal end. Furthermore, this immunity is not transmitted; indeed, the children of leprous mothers are quite susceptible to infection.

The nature of this acquired immunity is unknown. Probably immune opsonins for the bacilli are of most importance; complement-fixing antibodies have been found in the blood by some observers and allergic sensitization is said to occur as in tuberculosis.

Vaccine and Serum Treatment.—Various sera have been employed, but with indifferent or negative results. Probably the oldest method is that of Carrasquilla,⁵ who immunized horses with the sera of leprous individuals. A large literature has accumulated on the results of treatment with this supposedly immune serum, but the results are probably not better than those to be obtained with normal horse-serum and other non-specific agents.

¹ New York State Jour. Med., 1910, 236.

² Jour. Amer. Med. Assoc., 1911, 56, 1379.

³ Deutsch. med. Wchn., 1920, 46, 209.

⁴ Studies from the Roy. Vict. Hosp., 1906, 2, 1.

⁵ Wien. klin. Wchn., 1897, Nos. 41 and 42.

Immune sera have also been prepared by Sugai¹ and others by immunizing horses with the expressed juices of leprous nodules; Currie, Clegg, and Holman² have immunized with an acid-fast bacillus regarded as *Bacillus lepræ*. None of these sera can be said to have proved of value. Janin³ has used injections of blister serum from the patient, giving 8 to 10 c.c. by subcutaneous injection every ten days. Improvement was claimed for some cases.

A large number of vaccines have also been employed including the juices of ground up and expressed leprous nodules containing large numbers of bacilli; vaccines of various acid-fast and chromogenic bacilli regarded as the leprosy bacillus and autolytic extracts of these prepared after the manner of Koch's old tuberculin. Favorable results were claimed in some instances, but the vaccines were ordinarily prone to produce abscesses, and the consensus of opinion is to the effect that they have not proved beneficial.

A large literature, however, has accumulated on the use of nastin in treatment. Schumacher,⁴ Peiper,⁵ Scott,⁶ and others believe that the results are beneficial and encouraging, the latter observing improvement in 85 per cent. of cases. Wise and Minett⁷ observed temporary improvement in some cases only, while Minett⁸ and others have reported negative results.

Tuberculin has also been employed in treatment, but with generally negative results.

TREATMENT OF DISEASES OF THE EAR AND MASTOID

Furunculosis of the external auditory canal is practically always a staphylococci infection, *Staphylococcus pyogenes albus* being found in the small superficial lesions and *S. aureus* in the deeper lesions. In chronic and recurrent infections vaccine therapy with stock or autogenous staphylococcus vaccines is frequently of great benefit. Dosage and administration have been previously discussed under Furunculosis.

Vaccine Treatment of Acute and Chronic Otitis Media.—Otitis media is usually an ascending infection from the nasopharynx by way of the eustachian tube. Cultures generally show the presence of a single organism, but two or more may be the etiologic agents. Streptococci, pneumococci, and *Staphylococcus aureus* are most frequently found; the Friedländer bacillus, *Micrococcus catarrhalis*, and the influenza bacillus are found occasionally.

After rupture of the tympanum and in chronic otitis media other organisms may gain access through the external auditory canal and in cultures overgrow the primary organisms. Diphtheroid bacilli, *Bacillus pyocyaneus*, *B. proteus*, *B. coli*, and staphylococci are commonly found, usually in combinations of two or more different organisms.

In acute otitis media it would appear advisable to make cultures of the pus immediately after incision or rupture of the typanum; blood-agar is the medium of choice. These cultures generally disclose the primary organism in pure culture.

In chronic otitis media cultures should be made from the middle ear after cleansing of the external auditory canal in order to avoid as far as possible picking up secondary organisms. The pus should be streaked over

¹ Arch. f. Dermat. and Syph., 1912, cxii, 88.

² Lepra, 1912, 13, 25.

³ Rev. d. Med. et d'Hyg. Trop., 1913, 10, 81.

⁴ Arch. f. Schiffs u. Trop.-Hyg., 1913, 17, 15.

⁵ Arch. f. Schiffs u. Trop.-Hyg., 1913, 17, 183.

⁶ Indiana Jour. Med. Res., 1913, 1, 352.

⁷ Jour. Trop. Med. and Hyg., 1912, 15, 259.

⁸ Brit. Guiana Med. Ann., 1913, 24.

plates of blood-agar in order to permit the less hardy and slower growing but more important organisms a chance to multiply, especially pneumococci and streptococci. This is of considerable importance in relation to treatment with autogenous vaccines, inasmuch as vaccines of the more rapidly growing secondary and frequently saprophytic bacteria are of little use.

In *acute otitis media* more observers have reported prompt relief of pain and resolution of the inflammatory process following the early subcutaneous injection of a mixed stock vaccine of streptococci, pneumococci, and staphylococci.

In *chronic otitis media* autogenous vaccines are frequently of decided help in treatment while the usual local measures are being employed. It is essential to have very carefully prepared cultures; much of the success depends primarily upon the bacteriologic work. I believe that it is a good routine practice to institute vaccine therapy in all subacute cases before chronicity and involvement of the bony tissues is established. Even in the long-standing cases good results are sometimes observed from autogenous vaccine therapy, although a long series of injections are usually required, as is true of superficial infections in general.

In the otitis media of scarlet fever Weston and myself¹ observed that in general terms the suppuration was of shorter duration among the vaccine-treated cases; in some instances particularly good results were observed, although it is to be borne in mind that the disease is frequently cured in an equally short time by the usual forms of local treatment. Coates,² Haughey,³ and others have reported favorably upon the treatment of otitis media, especially with autogenous vaccines. They have proved especially useful in children, among whom local treatment is frequently difficult.

My practice is to prepare a vaccine for children up to twelve years of age containing a total of 500,000,000 organisms per cubic centimeter. Diphtheroid bacilli and *Bacillus proteus* are not included because they are generally saprophytes. *B. pyocyaneus*, however, is always included, and sometimes when found in pure culture in chronic infections has yielded satisfactory results. The organisms chosen for the vaccine are employed in equal proportions. For individuals over twelve years of age the vaccine is made to contain 1,000,000,000 per cubic centimeter.

The first dose is 0.1 c.c. by subcutaneous injection; subsequent injections are given at intervals of five to seven days in gradually increasing amounts until 1 c.c. is being given at one time. As a general rule six to eighteen injections are required, but if there is not distinct improvement after eight to ten injections I believe it is good practice to discard the vaccine and prepare a second.

If autogenous vaccines are not to be had, good results may be obtained with a stock vaccine of streptococci, pneumococci, and staphylococci; indeed, they sometimes yield better results than improperly prepared autogenous vaccines.

Rauch⁴ and Lawner⁵ have reported good results in the treatment of acute and chronic otitis media with intramuscular injections of 5 c.c. of market milk boiled for ten minutes and cooled. Gomperz,⁶ however, did not observe beneficial effects with this therapy. Hirsch⁷ employed subcutaneous injections of turpentine (20 per cent. dilutions), with negative results.

¹ Amer. Jour. Med. Sci., 1911, 142, 403.

² Jour. Amer. Med., Assoc., 1915, 65, 356; *ibid.*, 1917, 68, 162.

³ Ann. Otol., Rhinol., and Laryngol., 1915, 24, No. 1.

⁴ Wien. klin. Wchn., 1917, No. 43.

⁵ Wien. klin. Wchn., 1917, 30, 17.

⁶ Wien. med. Wchn., 1917, 67, No. 37. ⁷ Arch. f. Ohrenh., 1919, cv, 62.

In *tuberculosis* of the ear the subcutaneous injection of tuberculin frequently yields excellent results, and in the opinion of Randall and other otologists of extensive experience should be included in the plan of treatment. This subject will be discussed in more detail in the succeeding chapter on *Tuberculosis Therapy*.

Vaccine Treatment of Acute and Chronic Mastoiditis.—In the opinion of some physicians the prompt administration of a stock vaccine of staphylococci, streptococci, and pneumococci may shorten the course of acute mastoiditis and relieve the pain; the writer has had no personal experience with this therapy. Davis¹ has recently advocated the administration of stock vaccines of staphylococci and streptococci in acute mastoiditis complicating otitis media along, of course, with local treatment. He believes that the prompt institution of vaccine therapy reduces the percentage of cases requiring operation.

In chronic suppurative mastoiditis following operation autogenous, vaccines are worthy of trial, although in my experience they have not yielded encouraging results. Superficial infections of bony tissue are generally refractory to immunotherapy.

If vaccines are prepared great care should be exercised in the bacteriologic work in order to secure the streptococci or pneumococci, which are usually the organisms of primary etiologic importance.

TREATMENT OF DISEASES OF THE RESPIRATORY ORGANS AND ACCESSORY SINUSES

Skin tests for the diagnosis and treatment of allergic rhinitis, hay-fever, and asthma, and the treatment of diphtheria and pneumonia have been previously described.

Vaccine Treatment of Acute Rhinitis.—The uncertain state of our knowledge of the etiology of the "common cold" has been discussed on p. 815. According to some observers the organisms to be found in the nasal secretions by ordinary cultural methods are to be regarded as the etiologic factors, and especially pneumococci, streptococci, *Micrococcus catarrhalis*, staphylococci, and *B. influenza*; Tunncliffe has described a small anaërobic bacillus, and Foster, Kruse, and others a filterable virus.

It is obvious that until the etiology is conclusively determined that treatment of acute rhinitis with vaccines of the cultivatable bacteria has little or no standing in so far as specific therapy is concerned. On the other hand, it has been the experience of many physicians that the prompt subcutaneous injection of these mixed vaccines has served to abort or shorten the attacks of acute rhinitis. Stock vaccines have been commonly employed.

The writer has observed that the administration of autogenous vaccines of this kind prepared of cultures made during a previous attack has frequently been followed by a prompt relief of the symptoms of acute rhinitis in some cases; in the treatment of the rhinitis of scarlet fever, particularly dangerous because favoring the presence and dissemination of the scarlet fever virus, Weston and the writer² found autogenous vaccine of aid in shortening the course of the infection in some cases. Good results in the treatment of "common colds" with autogenous and stock vaccines have been reported by Fisher,³ Rowlette,⁴ and others.

¹ Penna. Med. Jour., 1922, 25, 306.

² Amer. Jour. Med. Sci., 1911, 142, 403.

³ Boston Med. and Surg. Jour., 1913, 168, 834.

⁴ Brit. Med. Jour., 1915, 1, 1046.

Whether or not the effects of vaccine therapy in the treatment of rhinitis are purely non-specific cannot be stated; the writer believes, however, that bacteria of the pyogenic group to be found in the thick nasal discharges are at least important from the standpoint of secondary infection, even though their significance as primary etiologic factors is uncertain. Furthermore, there is evidence indicating that these vaccines apparently possess some prophylactic value for some individuals (p. 816), and the whole subject is worthy of further investigation in view of the high incidence and importance of the disease.

Vaccine Treatment of Hay-fever.—Hay-fever is generally regarded as primarily due to allergic sensitization to various pollens; skin tests for diagnosis and desensitization by subcutaneous injections of pollen extracts have been previously described.

During the actual attack of hay-fever, however, the injection of extracts of the pollen or pollens responsible for the sensitization and the attack may afford slight or no relief.

In these cases Scheffegrell¹ and others have employed a mixed treatment of vaccine and pollen extract. Stock vaccines of staphylococci, pneumococci, *Micrococcus catarrhalis*, etc., have been generally employed. Injections are given at intervals of one or two days until the severity of the attack subsides. The pollen extract is then used, the vaccine injections being resumed if a severe paroxysm develops. In 3 per cent. of a series of 1000 cases Scheffegrell states that the treatment was limited to vaccine therapy alone and that the results in general were satisfactory when given in the acute attacks.

The only logical basis for vaccine treatment in hay-fever aside from the possible beneficial non-specific effects is to combat secondary bacterial infection facilitated by the changes in the mucous membranes instituted by the allergic reaction. It would appear that in some cases this secondary infection may be worthy of treatment with vaccines as indicated. I have noted beneficial effects in cases treated with autogenous vaccines, and especially those in whom the attacks were very severe and accompanied by asthma.

Chronic Rhinitis; Ozena.—In chronic or frequently recurring rhinitis the administration of autogenous vaccines are sometimes of distinct value in treatment before hypertrophic or atrophic changes have occurred. Cultures on blood-agar usually show the presence of one or more different organisms, and especially staphylococci, streptococci, pneumococci, *Micrococcus catarrhalis*, and Friedländer's bacillus. In my experience the administration of autogenous vaccines in cases of frequently recurring rhinitis in whom a state of chronicity may be said to have been established, has frequently yielded very good results. The vaccine is prepared of equal numbers of the bacteria secured in pure culture from plate cultures, employing blood-agar in order to favor the growth of the less easily grown organisms so that each cubic centimeter contains 1,000,000,000. Treatment is begun with 0.1 or 0.2 c.c. by subcutaneous injection and the injections given at intervals of five to seven days in increasing amounts. If improvement results after three to six injections, the intervals of injection is raised to two weeks, then once per month, and finally once in two to four months.

Vaccine therapy has been extensively employed in the treatment of *ozena*. The vaccines of the ordinary bacteria recovered in cultures of the fetid secretions or of the mucosa beneath the crusts have failed to establish their value in the treatment of this exceedingly disagreeable affection. Vaccines of the cocco-bacillus of Perez,² however, are claimed to have

¹ Hay-fever and Asthma, Lea & Febiger, 1922, 225.

² Ann. de l'Inst. Pasteur, 1899, 13, 937.

proved of value by Hofer,¹ Skillern and Holmes,² Horn,³ Klenk,⁴ Guggenheim,⁵ and others. The bacillus is rather difficult to cultivate and isolate in pure culture, so that stock polyvalent vaccines have been generally employed. The available reports indicate that it may be worth while using a vaccine of this bacillus, although its etiologic relationship to the disease cannot be said to have been definitely proved. Hofer regards the organism (*Bacillus ozæna-fatidæ*) as related to the Friedländer bacillus, while Horn claims that it is related to *B. bronchisepticus*, regarded by Ferry and others as the course of canine distemper. Perez found the bacillus in 8 of 22 cases of ozæna; in a recent study of 50 cases Ward⁶ found it in 22 cases.

An effort should be made to prepare an autogenous vaccine to contain 1,000,000,000 per cubic centimeter; the initial dose may be 0.1 c.c. by subcutaneous injection, and subsequent doses increased at intervals of five to seven days until some focal reaction occurs in the nose after each injection. A long series of injections are ordinarily required in conjunction with the usual local measures.

Vaccine Treatment of Acute and Chronic Sinusitis.—Infections of the accessory nasal sinuses are usually secondary to nasal and postnasal disease; in my experience pneumococci are the most frequently found organisms in smears and cultures, but staphylococci and streptococci also occur in many cases.

In acute sinusitis the prompt administration of a stock vaccine of staphylococci, streptococci, and pneumococci in total dose of 100,000,000 is reported by Davis⁷ and others to afford prompt relief in a large number of cases. Second and third injections are commonly given at intervals of forty-eight hours.

In chronic sinusitis (frontal, ethmoidal, sphenoidal, antral) autogenous vaccines are sometimes of value as part of the treatment. A special effort should be made, however, to obtain the cultures of pus with as little contamination as possible and preferably upon plates of blood-agar in order that the growth of pneumococci and streptococci so readily overgrown by staphylococci and diphtheroid bacilli may be obtained. The vaccine may contain a total of 1,000,000,000 per cubic centimeter and treatment begun with 0.1 c.c. Subsequent injections may be gradually increased at intervals of five to seven days until 1 c.c. is being given at one time. Slight focal reactions (increased hyperemia and discharge) are desirable; for this purpose intramuscular injections of large doses are sometimes more efficacious than subcutaneous injections.

Vaccine Treatment of Acute Tonsillitis, Pharyngitis, and Laryngitis.—Some physicians have observed good results from the subcutaneous injection of stock vaccines of staphylococci, pneumococci, streptococci, etc., early in the course of acute tonsillitis, pharyngitis, and laryngitis. Cultures usually show the presence of staphylococci or streptococci or a combination of these. The initial doses are small, varying from 25,000,000 to 100,000,000 of each organism per cubic centimeter; second and third injections are given at intervals of forty-eight to seventy-two hours.

Vaccine Treatment of Chronic Bronchitis.—In chronic bronchitis the administration of autogenous vaccines frequently proves of great value as an adjuvant to treatment, and especially in cases without advanced bronchiectasis and emphysema. In my experience the results have been among

¹ Wien. klin. Wchn., 1913, 25, 1011.

² New York Med. Jour., August 15, 1908.

³ Jour. Amer. Med. Assoc., 1915, 65, 788.

⁴ Missouri State Med. Jour., 1916, 13, 197.

⁵ Interstate Med. Jour., 1915, 22, 2.

⁶ Jour. Infect. Dis., 1916, 19, 153.

⁷ Penna. Med. Jour., 1922, 25, 306.

the most satisfactory in vaccine therapy. Cases of bronchitis due primarily to passive congestion from cardiac decompensation are not included in this group. Those due primarily to bacterial infection from a preceding attack of influenza, pneumonia, or repeated attacks of acute bronchitis and displaying what Johnson has called the "pneumocatarrhal diathesis," are to be selected for vaccine therapy. In Chapter XXXII the work of Walker with bacterial vaccines in chronic bronchitis and asthma has been discussed, especially in relation to allergic asthma; Gillett,¹ Pirie,² Babcock,³ and others have reported favorably upon the use of autogenous vaccines in chronic bronchitis and asthma.

Cultures on blood-agar or other suitable medium should be made of the bronchial secretions, and preferably of that coughed up upon arising in the morning. As a general rule streptococci predominate, although this varies in different cases, and staphylococci, pneumococci, *Micrococcus catarrhalis*, the Friedländer bacillus, and diphtheroid bacilli may be found. Smears of the secretions should be examined at the same time, as in this manner an idea may be gained regarding which organism predominates.

Skin tests may be conducted to determine if allergy exists for any of the organisms secured in pure culture; the method has been described on p. 684. If asthma is absent these tests, however, are apt to be negative or indefinite, in which case mixed vaccines are to be employed. If positive skin reactions are observed the vaccine may be prepared of the organism or organisms producing the positive reactions.

My practice is to prepare a vaccine containing 1,000,000,000 per cubic centimeter if more than one organism is used in equal proportions. If plates and smears show a great preponderance of streptococci these alone are employed; diphtheroid bacilli, *Bacillus proteus*, fungi, and the like, which may be present by reason of contamination of the bronchial secretions with saliva, are not included.

The initial dose is 0.1 c.c. by subcutaneous injection; subsequent doses are gradually increased and given at intervals of five to seven days. Doses large enough to produce slight focal reactions characterized by increased expectoration during the twenty-four hours succeeding an injection are advisable, and especially with the first three or four doses.

It is not well to give more than six to ten doses of the same vaccine unless decided improvement is noted; a fresh vaccine should be prepared rather than one vaccine continued over a long period of time.

Treatment of Asthma.—The diagnosis of allergic asthma by skin tests has been described on p. 666; likewise treatment of allergic asthma including that type due to allergic sensitization to the proteins of bacteria present in the secretions. Walker,⁴ Sicard,⁵ Babcock,⁶ Hutcheson and Budd,⁷ and others have reported very favorable results in the treatment of some cases of bacterial asthma with vaccines. The latter authors have simply planted 1 c.c. of washed sputum in 10 c.c. of broth to which is added a few drops of sterile guinea-pig serum. After forty-eight hours' incubation the culture is heated to 60° C. for two hours, preserved with phenol, and injected subcutaneously as a vaccine, beginning with 1 minim and gradually increasing at weekly intervals until 15 minims are being given at one time. Of 20 cases treated in this manner, 12 secured complete relief after one to five injections, 5 showed improvement, 2 no improvement, and 1 became worse.

¹ Brit. Med. Jour., 1913, 1, 387.

² Brit. Med. Jour., 1913, 2, 1268.

³ Jour. Amer. Med. Assoc., 1915, 65, 1942.

⁴ Arch. Int. Med., 1919, 23, 220.

⁵ Amer. Jour. Med. Sci., 1917, 153, 856.

⁶ Jour. Amer. Med. Assoc., 1915, 65, 1942.

⁷ Amer. Jour. Med. Sci., 1918, 155, 826.

Various non-specific agents have likewise been employed. Auld¹ has used Witte's *peptone* by intravenous and subcutaneous injection, the dose being 5 to 10 c.c. of a sterilized 5 per cent. solution in saline solution. Sharp reactions usually follow and great care must be exercised. Some cases are promptly benefited by one or two injections; others are relieved by injections at intervals, and in others no beneficial effects are observed. Pagniez² and Widal, Abrami and Brissand³ have administered peptone by mouth. Kahn and Emsheimer have reported definite improvement in 6 cases treated with subcutaneous injections of the patient's *defibrinated blood* in dose of 20 to 30 c.c. by subcutaneous injection at intervals of one week for ten injections.

Danyisz⁴ has employed injections of his "*enterovaccine*" described on p. 994. Miller and Petersen have employed *intravenous injections of typhoid bacilli* and report that in some cases the results were quite satisfactory; in others there were no apparent effects, and especially those in which food sensitization was demonstrable.

Treatment of Pleuritis (Empyema).—Acute pleurisy with empyema is generally a pneumococcus infection developing during lobar pneumonia or a streptococcus infection following bronchopneumonia. After drainage has been established, secondary infection with staphylococci, diphtheroid bacilli, and *B. pyocyaneus* may occur. Chronic primary pleurisy is usually a tuberculous infection; other organisms may gain access from cavities and bronchi and produce secondary infections.

Treatment of acute pneumococcus and streptococcus pleuritis with intrapleural injections of *antipneumococcus* and *antistreptococcus sera* has not been generally employed, although encouraging results have been observed in the treatment of experimentally produced pleuritis of the lower animals by injections of these sera. Floyd⁵ has treated 20 cases of pneumococcus pleuritis and empyema produced during pneumonia by Types I and II pneumococci, with intrapleural injections of Type I or Type II serum. In each case thoracotomy was performed for drainage, the pus examined, and the pneumococci typed. Subsequently, every other day, when it was possible, the chest cavity was irrigated with saline solution, and 4 ounces of equal parts of appropriate immune serum and saline solution were put into the chest. It was found that healing occurred much more rapidly if secondary invaders, as staphylococci and diphtheroid bacilli, could be excluded; the serum therapy appeared to aid in phagocytosis and to shorten the course of the infection.

Vaccines have been employed, with indifferent results. The writer is of the opinion that cultures should always be made and treatment with autogenous vaccines instituted as part of the treatment of pneumococcus, streptococcus, and staphylococcus pleuritis along with adequate drainage. These vaccines are so prepared that each cubic centimeter contains 1,000,000,000 organisms; the initial dose is 0.1 c.c. by subcutaneous injection. Subsequent injections are given at intervals of five to seven days and gradually increased until 1 c.c. is being given at one time.

Autoserum therapy, consisting of the withdrawal of 5 to 10 c.c. of fluid and injecting at once subcutaneously, has been advocated by a number of investigators for the treatment of *tuberculous* pleurisy.

Numerous investigators, as, for example, Gilbert,⁶ Macon,⁷ Schnütgen,⁸

¹ Brit. Med. Jour., 1918, 2, 49.

² Presse méd., 1920, 28, 65.

³ Presse méd., 1921, 29, 181.

⁴ Arch. Int. Med., 1916, 18, 445.

⁵ Jour. Immunology, 1920, 5, 321.

⁶ Gaz. des Hôp., 1894, 560.

⁷ La Presse Médicale, 1909, No. 71, 627.

⁸ Berl. klin. Wchn., 1909, No. 3, 97.

Fishberg,¹ Pfender,² Dodal,³ and others, have reported favorable results in the treatment of *tuberculous pleurisy with effusion*, cases that arise either insidiously or abruptly with pain in one already tuberculous, or in one in whom tuberculosis is suspected, following withdrawal of a portion of the fluid and immediate injection of from 2 to 5 c.c. into the subcutaneous tissues. In these cases there is usually a sharp reaction, consisting of a rise in temperature, occasionally accompanied by chill, lassitude, and, in the majority of cases, diuresis or, more rarely, diarrhea, followed by gradual absorption of the fluid within the following few days up to two or three weeks. Fishberg mentions the disappearance of pain, dyspnea, and prostration within two or three days in favorable cases.

It is difficult to state whether the improvement is due to autotherapy or simply to the puncture and removal of so much fluid. Eisner⁴ has seen a leukocytosis follow injection of serum in experimental tuberculous infections of rabbits and guinea-pigs, and believes that this explains the good results in this particular form of therapy. Zimmermann⁵ has expressed a similar opinion. Other investigators assert their belief in the presence of aggressins, bacteriolytic amboceptors, complements, and endolysins from disintegrated leukocytes as explaining the results. It is more likely that these fluids contain the bacilli or their products, and constitute a form of vaccine or autotuberculin, stimulating body cells to produce antibodies largely in the nature of bacteriotropins and bacteriolysins. Levy, Valenzi and Ponzin,⁶ Szurek,⁷ and Arnsperger⁸ are inclined to believe that the beneficial results are obtained independently of the injections, and while the procedure is quite generally regarded as perfectly safe, Jousset⁹ has recorded a case of cold abscess following an injection. This mode of treatment seems to have failed in about 10 to 15 per cent. of cases. Lyter¹⁰ has recently reviewed the subject and has come to the conclusion that the injections have little or no influence in hastening absorption of the exudate. In 23 cases 8 were completely absorbed in two weeks' time—34 per cent., while in the balance the effusion did not lessen as a result of the treatment. Lyter observed practically no leukocytosis, and in only 2 of the 8 rapidly absorbing cases did he observe any increase of temperature.

The *technic* is very simple, and the injections may be given by any physician who can make an ordinary exploratory puncture. In all cases where puncture shows the presence of a serous fluid the needle should not be withdrawn completely, but when its point has reached the subcutaneous tissues, from 2 to 5 c.c. should be injected then and there. In some patients it will be necessary to repeat the treatment several times every two or three days before any effect becomes evident.

In view of the fact that the fluid may contain a sufficient number of living tubercle bacilli to produce secondary infection, it would appear advisable to withdraw fluid into an equal volume of 2 per cent. sodium citrate in normal salt solution, heat at 60° C. for one-half to one hour, and preserve in a sterile container with the addition of a few drops of 5 per cent. phenol until ready for subcutaneous injection in doses of from 2 to 5 c.c.

¹ Jour. Amer. Med. Assoc., 1913, lx, 962.

² Wash. Med. Ann., 1914, xiii, 83.

³ Wien. med. Wchn., 1910, 455.

⁴ Zeitschr. f. klin. Med., 1912, lxxvi, 34.

⁵ St. Petersb. med. Wchn., 1909, No. 34, 461.

⁶ Bull. et. mem. de la Soc. d. hôp. d. Paris, 1910, xxvii, 265.

⁷ Med. Klinik, 1909, No. 44, 1665.

⁸ Therap. d. Gegenwart, 1911, lii, 495.

⁹ Arch. gén. de méd., 1912, xci, 141.

¹⁰ Amer. Jour. Med. Sci., 1918, 156, 665.

TREATMENT OF DISEASES OF THE DIGESTIVE TRACT

The biologic therapy of *typhoid fever*, *bacillary dysentery*, and *Asiatic cholera* has been previously considered in this chapter.

Vaccine Treatment of Chronic Gingivitis and Alveolitis; Apical Abscesses and Focal Infection.—Chronic gingivitis and pyorrhea alveolaris (Rigg's Disease) are frequently bacterial infections in which different kinds of streptococci, pneumococci, staphylococci, etc., are to be found in the pus. Endamœba buccalis are frequently found in smears, and are regarded by Smith and Barret as bearing a secondary relationship to the disease, that is, these protozoa are not the primary cause of gingivitis, but by reason of their burrowing movements may mechanically carry bacterial infection into the deeper tissues of the gums and even to the periosteum of the alveolar processes or tooth sockets just as Endamœba histolytica are known to do in the wall of the colon in amebic dysentery. In a large number of cases of this disease smears reveal a great increase of various spirochetes sometimes associated with the banana-shaped or fusiform bacillus of Vincent's angina. This type of spirochetic gingivitis usually responds to the local application of 1 per cent. solutions of arsphenamin, and the subject will be discussed again under Chemotherapy.

In those cases of gingivitis in which smears of the secretions removed from the pockets about the teeth show only a few spirochetes and enormous numbers of bacteria with or without amebas, autogenous vaccines may prove of value as an adjuvant to the usual treatment applied to the gums.

Cultures should be made on blood-agar in order to facilitate the growth of streptococci and staphylococci, which may be overgrown by staphylococci, diphtheroid bacilli, and other organisms. After cleansing the surface of the gums the pus expressed from the pockets is especially to be cultured. Head employs the following method: The pocket from which the material is to be obtained is first protected from mouth contamination by a sterile napkin. The gingival margin of the pocket is then washed with sterile cotton dipped in sterile salt solution. The margin of the pockets are slightly seered with an electrocautery so that the gum is distinctly whitened. The root of the tooth adjacent to the margin is thoroughly gone over with the cautery to destroy all extraneous flora of the mouth which may cause contamination. A thin spear of platinum about 3/1000 inch in thickness is then heated to a cherry-red color and plunged into the bottom of the pocket. It is particularly essential that this blood-serum should be obtained from the wall of the pocket, so that any bacteria lurking within the tissues of the pocket may be secured, as it is presumable that the germs within the walls are most responsible for the disease. The smear should then be drawn directly out without touching any portion of the mouth to prevent contamination and the material inoculated in tubes of blood-agar or other suitable medium in the usual manner.

The vaccine should be a mixed one if more than one organism is recovered in the pus, but streptococci and staphylococci are of most importance. The writer prepares these vaccines to contain 1,000,000,000 per cubic centimeter; the first dose is 0.1 c.c. by subcutaneous injection. Subsequent doses are given at intervals of five to seven days and in gradually increasing amounts.

Head,¹ who has had an exceptional experience in the vaccine therapy of gingivitis, commences treatment with much smaller doses, as low as 30 000 organisms. This dose is raised weekly as long as the patient bears

¹ Medical Record, July 6, 1918.

the injections well, even if the dose goes up to 1,000,000,000. As long as improvement is noted under a dose, that dose is maintained.

Goodby¹ was probably first to apply vaccine therapy in gingivitis. Beebe,² Eyre and Payne,³ Williams,⁴ Beebe,⁵ Medalia,⁶ and others have made favorable reports, and in the bacterial forms of the disease vaccine therapy appears worthy of trial as part of the treatment.

Aside from the possible value of vaccine therapy as an aid in the treatment of the purely local infection of the gums and alveolar tissues, these vaccines may possess an important prophylactic and curative activity for systemic infections due to the distribution of bacteria from these foci. This is especially true when abscesses are found at the roots of teeth. It is not an uncommon experience to observe that individuals with these infections may develop acute exacerbations of arthritis, iritis, neuritis, endocarditis, etc., as a result of the extraction of teeth or surgical treatment of the infected gums, probably caused by the introduction of extra amounts of bacteria or their products into the blood-stream from these *focal infections*. Reactions of this kind appear to show indubitably a direct relation between the local infection of the gums and tooth sockets and the distant lesions, and in these cases at least the employment of an autogenous vaccine appears a logical procedure not only for the aid it may render in the treatment of the local infection, but, even more importantly, for its possible value in the prophylaxis and treatment of the secondary infections. Daland⁷ has emphasized the necessity of careful search for primary foci of infection in *ulcerative endocarditis* and especially the relation of oral sepsis to this infection. He recommends the removal of the foci if at all possible and treatment with autogenous vaccines, usually of *Streptococcus hemolyticus*.

Focal infections of the upper respiratory tract embracing the nose and tonsils as well as the gums are regarded by Rehfuss and others as bearing an important relation to some bacterial infections of the gastro-intestinal tract. The investigations of Rosenow, previously referred to in a discussion of the subject of Focal Infection, indicate that streptococci from these foci may be productive of gastric ulcer, cholecystitis, and appendicitis. For these reasons more and more attention is being paid the important relation of "oral sepsis" to diseases of the gastro-intestinal tract, as well as of more distant organs, and autogenous vaccines of the bacteria of the primary foci are being more widely employed as part of the general plan of treatment.

Treatment of Cholecystitis and Hepatitis.—Until recently vaccine therapy has had no wide application in the treatment of bacterial infections of the gall-bladder and its ducts and the biliary ducts of the liver. Stock vaccines have been administered in some cases prepared of streptococci, colon bacilli, etc., but without preliminary bacteriologic examinations. Autogenous and stock vaccines have also been employed in the treatment of these infections after drainage or removal of the gall-bladder.

By means of Meltzer-Lyon method of gall-bladder drainage it is now possible to secure specimens of bile for microscopic and cultural study. The writer has had the opportunity of examining a large number of specimens for Dr. Lyon and his former associate Dr. Bartle, the technic and findings

¹ Lancet, 1907, 1, 663; *ibid.*, 1909, 2, 1875.

² Boston Med. and Surg. Jour., 1909, cxi, 613.

³ Proc. Roy. Soc. Med., Odontological Sec., 1909, 3.

⁴ Amer. Jour. Med. Sci., 1911, 141, 666.

⁵ Boston Med. and Surg. Jour., 1909, cxli, 613.

⁶ Dental Cosmos, January and February, 1913; Boston Med. and Surg. Jour., 1913, clxix, 786.

⁷ Medical Clinics of North America, Saunders Co., September, 1917.

being reported elsewhere.¹ Staphylococci, colon bacilli, and streptococci are commonly found, and the administration of autogenous vaccines has rendered important aid in the treatment of some cases of infection of these organs, especially vaccines of the staphylococci and streptococci. It is our practice to prepare these autogenous vaccines to contain 2,000,000,000 organisms per cubic centimeter. The first dose is 0.1 c.c. by subcutaneous injection. Subsequent doses are given at intervals of five to seven days and gradually increased.

Mention may also be made here of *autoserum* treatment of hepatitis with ascites. This therapy, first introduced by Gilbert for the treatment of pleurisy with effusion, has also been employed by Audibert and Monges,² Vitry and Sezary,³ Lahiri,⁴ and others for cirrhosis of the liver with ascites. The technic consists of the subcutaneous injection of 3 to 5 c.c. of the patient's own sterile ascites fluid at intervals of five to seven days. The above-mentioned observers report an increased output of urine and gradual absorption of the transudate. The beneficial results ascribed to this treatment are probably due to the effects of a non-specific protein therapy.

Vaccines have also been employed in the treatment of *colitis*, chronic *intestinal toxemia*, *appendicitis*, etc. Drew⁵ reports that in 14 out of 15 cases of membranous colitis smears and cultures showed a great preponderance of *Streptococcus fecalis*. Autogenous vaccines beginning with small doses were found of aid in treatment. Langeron⁶ has reported a case of sepsis due to *Streptococcus fecalis* (*enterococcus*) successfully treated with an autogenous vaccine. Satterlee⁷ states that the predominating factor in the symptomatology of chronic intestinal toxemia is the colon bacillus and that autogenous vaccines of this bacillus should be administered as part of the treatment to immunize against the toxic substances being produced. Of interest in this connection is the work of Torrey and Rahe,⁸ who found that in a number of dogs it was possible to effect a temporary suppression of a certain variety of *Bacillus coli* normal to the intestinal tract by injections of vaccine. Autogenous vaccines in large doses are apparently necessary for bringing about a decrease in numbers of the colon bacilli with a coincident use of specific antibodies in the blood.

Vaccine Treatment of Sprue.—Michel⁹ has recently reported the successful treatment of sprue with vaccines prepared of a monilia (*Monilia psilosis*), which Ashford has isolated from the tongue and feces of individuals with this disease and regards as the etiologic agent.

Michel has described a method for preparing the vaccine which he administers in dose of 0.1 c.c. by subcutaneous injection; subsequent injections are given at intervals of ten days in gradually increasing amounts.

Of 81 cases diagnosed by Ashford and yielding *Monilia psilosis* in the feces and positive complement-fixation tests, 62 were treated with vaccine. Of these, 49 were discharged cured, 12 were improved, and 1 died. These results were regarded as very encouraging.

¹ Gall Tract Disease, its Diagnosis and Treatment, Lea & Febiger.

² Presse méd., 1910, 18.

³ Rev. d. méd., 1913, 33.

⁴ Practitioner, London, 1912, 88, No. 3.

⁵ Jour. State Med., London, 1918, 26, 217.

⁶ Bull. d. l. Soc. Méd. d. Hôp., 1918, 42, 43.

⁷ Jour. Amer. Med. Assoc., 1916, 67, 1729.

⁸ Jour. Immunology, 1920, 5, 133.

⁹ Jour. Infect. Dis., 1918, 22, 53.

TREATMENT OF DISEASES OF THE KIDNEY AND BLADDER

Vaccine Treatment of Bacteriuria.—Not infrequently bacteria are found in the urine collected under rigid aseptic precautions, without any evidences of local or systemic disturbances. In fully 50 per cent. of these cases the colon bacillus is found. Bacteriuria is more common in women than in men, as shown by the investigations of Willians and Murray¹ and others. Colon bacilli are likewise found in the urine of children, especially female children, and frequently without demonstrable evidences of pyelitis or other infection. How colon bacilli gain access to the urinary tract in apparently normal individuals is not definitely known; of course if catheterization has been practised this avenue of contamination is first suspected. It is probable that in some cases, and especially in women, contamination occurs by way of the urethra. Otherwise the entrance of colon bacilli into the blood from the intestinal tract and their elimination through the kidneys without the production of infection is to be suspected. Staphylococcic bacteriuria may also occur, but the majority of cases are due to colon bacilli. Of special interest in this connection is the possibility of elimination of tubercle bacilli in the urine without infection of the urogenital organs. It would appear that this is only possible in cases of tubercle bacilli in the blood and their ability to pass into the uriniferous tubules. Personally I doubt that this occurs, and in my experience the presence of tubercle bacilli in urine has always been found due to a tuberculous infection of the kidney or other organs of the urogenital tract.

Vaccines have been employed in the treatment of bacteriuria, but without much success. Whenever employed it is essential that autogenous vaccines be prepared of cultures of the urine secured by catheterization under rigid aseptic conditions. Many different strains of *Bacillus coli* are known to exist, and nothing is to be hoped for in vaccine therapy of colon infections unless the strain or strains present in the urine are employed.

Vaccine Treatment of Cystitis.—Bacterial infections of the bladder may occur as a result of ascending infections from the urethra and prostate, catheterization, and direct injury; doubtless, infection may descend from the kidneys and especially in tuberculosis.

Colon bacilli are frequently the primary etiologic factors, but streptococci, staphylococci, tubercle bacilli, and other organisms may be present either as primary infection or secondary invaders.

The vaccine treatment of tuberculous cystitis is considered in the succeeding chapter under Tuberculin Therapy. In other bacterial infections autogenous vaccines may prove of aid as part of the general and local treatment.

It is essential, however, that the urine for culture is collected under rigid aseptic precautions in order that the infecting bacteria are obtained. Billings,² Williams and Murray, and others have found colon vaccines of value; even better results have been observed in staphylococcic and streptococcic infections.

During the Great War, Walker and Shera found autogenous vaccines very useful in the treatment of paraplegic soldiers infected by rough-and-ready catheterization on the field and 90 per cent. of whom later developed cystitis due to *B. coli* or a staphylococcus, or a mixture of these.

Vaccines may be prepared containing 1,000,000,000 per cubic centimeter; the initial dose may be 0.1 c.c. by subcutaneous injections given at intervals

¹ Jour. Obst. and Gyn., Brit. Emp., 1912, 22, 65

² Amer. Jour. Med. Sci., 1910, 139, 625.

of five to seven days in increasing amounts. It is well to secure fresh cultures and prepare a second vaccine after six to ten doses have been given, instead of administering the same vaccine over a prolonged period.

Treatment of Pyelitis of Children.—This infection is almost invariably due to a strain of the colon bacillus and the route of infection usually obscure or unknown. The infection may be present without producing well-marked symptoms and is a troublesome infection from the standpoint of treatment.

Vaccines are apparently helpful in the treatment of some cases as reported by Freeman¹ and others; it is essential that autogenous vaccines be employed. Securing urine under proper conditions for cultures is frequently very difficult and requires special methods.

For children varying in age from one to twelve years the vaccine may contain 500,000,000 bacilli per cubic centimeter. The initial dose may be 0.1 c.c. by subcutaneous injection; subsequent injections are given at intervals of five to seven days in gradually increasing amounts.

Karo² has employed subcutaneous injections of *terpichin*; 20 per cent. solutions of *terpentine* in sterile olive oil may be injected subcutaneously in dose of a few drops to produce sterile abscesses and non-specific effects. The intramuscular injections of 1 c.c. of *milk*, boiled for ten minutes and cooled, is also worthy of trial.

Treatment of Pyelonephritis.—Pyelonephritis may be caused by *Staphylococcus aureus*, streptococci, tubercle bacilli, and other organisms during the course of bacteremia from some primary focus of infection, or develop from a pyelitis and ascending infection from the urinary bladder. In the latter cases the colon bacillus is usually the cause.

Vaccines have been employed in the treatment of some cases, but it is not possible to express an opinion of their value. It would appear that the consensus of opinion among surgeons is in favor of nephrectomy and especially in tuberculous nephritis. Bumpus³ has found that the administration of mixed colon bacillus vaccine in prostatic cases does not engender an immunity to renal infection preliminary to operations on the prostate and bladder.

If operation is refused or contraindicated it would appear worth while to employ an autogenous vaccine prepared of cultures of the urine secured by catheterization, along with general measures, and particularly a search for and removal of primary foci in suppurative nephritis of embolic origin.

Such vaccines may contain 1,000,000,000 organisms per cubic centimeter and treatment begun with the subcutaneous injection of 0.1 c.c.

Gow⁴ has reported 1 case of colon bacillus pyelonephritis successfully treated with intravenous injections of 50,000,000, 75,000,000, and 125,000,000 bacilli.

TREATMENT OF DISEASES OF THE EYE

Infection and Immunity in Diseases of the Eye.—Compared to the frequency of infection in other parts of the body infections of the eye after injuries and operations are singularly infrequent; undoubtedly the eye shows less tendency to infection than other parts of the body. This natural resistance is largely due to mechanical cleansing by the tears; these secretions also possess a feeble but relatively unimportant bactericidal activity.

Infections of the conjunctiva, lids, and lacrimal gland are usually due to direct implantation of organisms; the pneumococcus, gonococcus, staphylococcus, Koch-Weeks and Morax-Axenfeld bacillus are particularly important

¹ Amer. Jour. Dis. Child., 1913, 6, 117.

² Deut. med. Wchn., 1919, xvi, 266.

³ Jour. Amer. Med. Assoc., 1918, 70, 213.

⁴ St. Barth. Hosp. Jour., 1918-19, 26, 75.

in this connection and virulent strains are able to overcome the factors of natural resistance with the production of purulent inflammation. Even in severe streptococcus, pneumococcus, and other blood bacteremias these structures are only rarely involved by embolic infections. Why diphtheritic, gonococcus, pneumococcus, streptococcus, staphylococcus, and influenzal conjunctivitis are not more common in view of the frequent chances for direct inoculation of the conjunctiva and cornea from foci in the nose, throat, and other mucous membranes cannot be stated, unless the conjunctiva enjoys a peculiar tissue immunity acquired especially in adult life, aided by the protection afforded by the tears. Minute injuries of the conjunctiva and cornea favor infection, but these preliminary breaks in the epithelial barrier are not essential for infection. Apparently some organisms are able to secure a position on the intact epithelium and by means of toxic products induce suppurative changes. In this connection the recent experiments of Brown and Pearce¹ are of significance; these investigators have succeeded in producing syphilis of the eyes of rabbits by dropping *Treponema pallidum* into the apparently normal conjunctival sacs.

Infections of the cornea—keratitis—may occur by direct implantation or by way of the blood-stream. Ulcers—usually pneumococcic or streptococcic—are probably exogenous infections because the cornea is avascular and embolic abscesses can occur only at the margin. However, the *T. pallidum*, tubercle, and lepra bacilli usually infect the cornea through the blood and lymphatic channels, producing either diffuse (parenchymatous) or punctate keratitis, and it is possible, but not probable, that organisms of the pyogenic group (pneumococci and streptococci) may be brought to the margin of the cornea in the blood-stream and infiltrate the tissues by way of the lymphatics. This is especially true in panophthalmitis.

The cornea may also be involved from the rear, that is, by infection of the aqueous humor from a pre-existing iritis or iridocyclitis.

Infections of the iris, choroid, and retina are usually endogenous unless directly infected by injuries and operations. Panas originally believed that the toxins of bacteria rather than the bacteria themselves, were the primary etiologic agents. Most investigators at the present time, however, regard microbial infections of these tissues due to the presence of the microbes themselves; iritis especially is now known to bear a very important relation to focal infection. Streptococci, gonococci, and pneumococci from foci in the tonsils, teeth (apices), accessory nasal sinuses, urogenital organs, etc., are especially important in this relation. Experimental iritis has been produced by several investigators by the intravenous injection of bacteria in rabbits, but I do not know of the successful production of these lesions by injections of the toxic products of bacteria. However, the possibility of inflammation of the iris, choroid, and retina by toxins rather than by the bacteria themselves cannot be denied, and especially since we know that toxins may selectively locate in other tissues, notably the diphtheria toxins in the ganglia of the heart and tetanus toxins in the tissues of the spinal cord. The subject is a fertile field for further investigation.

Why bacteria as the gonococcus, *Diplococcus rheumaticus*, streptococci, gonococci, etc., locate in the iris, choroid, and retina is not definitely known. Severe blood infections with these and other bacteria are not infrequent and the eye escapes. Indeed, it is the rule for these infections to develop insidiously and one cannot escape the conviction that there is a strong possibility of infection by strains possessing a selective affinity for these tissues rather than infection on purely mechanical basis, as, for example, by embolic or

¹ Jour. Exper. Med., 1921, 34, 167.

vascular occlusion. This is especially true of the retina, where the vessels are rather large and not at all favorable to embolism.

For some unknown reason general blood or systemic infections from the eye are rare. Even in severe suppurative panophthalmitis blood-cultures are usually sterile. Fever, leukocytosis, etc., indicate the absorption of toxic substances in various eye infections, but blood infections with metastases to other organs are quite uncommon. The eye is not the primary seat of focal infection, indeed, it is usually the sufferer from primary foci situated elsewhere.

The immunity of the eye is largely cellular and aided by mechanical factors. Of course, antibodies in the blood are brought in contact with the vascularized tissues, but the humors and avascular cornea do not share in the protection afforded by humoral immunity unless vascularity is increased by injury followed by increased exudation of serum from hyperemic vessels. Furthermore, the lymph is decidedly bactericidal and in this manner tends to prevent infections of the cornea and offer some resistance when infection has occurred.

Serum Treatment of Pneumococcus Infections.—Axenfeld¹ has stated that the subcutaneous injection of 10 to 20 c.c. of antipneumococcus serum is of value for prophylactic immunization prior to cataract operations when pneumococci are present on the conjunctiva. We now know that the pneumococci found on the normal conjunctiva are usually non-virulent and belong to Type IV. On the other hand, it is not possible to sterilize the conjunctival sac without doing some injury, although something may be accomplished in this direction by preliminary instillations of solutions of boric acid. In this connection I may state that solutions of mercuriophen prepared by Schamberg, Raiziss and myself² are strongly pneumococcidal in 1 : 3000 to 1 : 6000 and do not produce irritation in dilutions as low as 1 : 500. This compound has proved particularly useful in connection with preoperative preparation of the eye as well as in the treatment of pneumococcus conjunctivitis.

If Type I pneumococci were found in the conjunctiva and particularly if associated with inflammatory changes, and operation were imperative, as in glaucoma or in penetrating injuries, the subcutaneous or intramuscular injection of 20 to 30 c.c. of Type I serum would appear to be a useful procedure for prophylactic immunization.

In the treatment of pneumococcus infections of the cornea (ulcus serpens), iritis, and in panophthalmitis, pneumococcus serum may prove useful in Type I infections; the subcutaneous injection of 10 to 20 c.c. of serum, however, is useless, as stated by Axenfeld. If used at all, 50 to 100 c.c. should be injected intravenously. In pneumococcus conjunctivitis and dacryocystitis serum therapy is not indicated. The most important pneumococcus lesion is corneal ulcer, and in severe Type I infections the intravenous injection of serum combined with local instillation may be of value. The instillation of 1 : 3000 to 1 : 6000 solutions of mercuriophen is of aid in infections with all types of pneumococci.

Serum Treatment in Streptococcus Infections.—Antistreptococcus serum has not been widely employed. Small doses (10 to 20 c.c.) by subcutaneous injection have been used in corneal ulcerations, but with negative results. In severe streptococcus infections, however, antistreptococcus serum may prove of value when given intravenously in doses of 50 to 100 c.c.

¹ Serumtherapie infektiöses Augenkrankungen, Freiberg, 1905. (Full bibliography to early literature.)

² Jour. Infect. Dis., 1919, 24, 1547.

Serum Treatment of Gonococcus Conjunctivitis.—The administration of antigonococcus serum may prove of aid in the treatment of both infantile and adult infections. For infants 10 c.c. of serum may be injected in the muscles of the buttocks when the infection is unusually severe. Corneal complications are less improved by serum than the conjunctivitis. In adults the serum should be given intravenously in doses of 50 to 100 c.c.

Serum Treatment of Diphtheric Conjunctivitis and Keratitis.—In acute diphtheria of the eye antitoxin should be given in large doses (10,000 to 20,000 units) by intramuscular injection; intravenous injection is preferable when conditions permit. Antitoxin may be instilled into the eye if a preservative free serum is available, otherwise the serum should be diluted with at least 3 parts of sterile saline solution to reduce the irritation caused by phenol or tricesol in the serum.

The administration of serum usually aids greatly in the treatment of the conjunctivitis, but the keratitis is much less affected, due in large part to secondary infection with pyogenic organisms.

Diphtheria antitoxin is of no value in *Bacillus xerosis* infections; on the other hand, these are usually chronic infections and serum therapy is not indicated.

Serum Prophylaxis of Tetanus of the Eye.—Tetanus only rarely develops from injuries of the eye, but in penetrating wounds contaminated with earth and dirt the subcutaneous injection of 500 to 1000 units of antitoxin has been advocated as a prophylactic measure.

Vaccine Treatment of Styes and Ulcerative Blepharitis.—These infections are staphylococcic and usually due to *Staphylococcus aureus*. After correction of eye strain, if such exists, the administration of an autogenous or stock staphylococcus vaccine is indicated and has generally yielded good results as reported by Mayou,¹ Medalia,² and others. The vaccine may contain 1,000,000,000 cocci per cubic centimeter and treatment begun with 0.1 c.c. by subcutaneous injection. Subsequent injections are given at intervals of five to seven days in increasing amounts until six to ten injections have been given. It is sometimes advisable to give an additional one or two injections several months later to reinforce immunity.

Vaccine Treatment of Chronic Dacrocystitis.—These infections in my experience are usually pneumococcic, but staphylococcic and streptococcic infections occur. Autogenous vaccines are frequently helpful when employed with local measures as reported by Bryan³ and others. The vaccine may contain 1,000,000,000 cocci per cubic centimeter and treatment begun with the subcutaneous injection of 0.1 c.c. Subsequent injections are given every five to seven days with increasing amounts. Local applications of 1 : 3000 mercuraphen are frequently very helpful.

Treatment of Pollen Blennorrhea (Hay-fever).—This condition usually develops during the spring months due to sensitization to various spring pollens; also in August, due to the pollen of ragweed and other plants. Blennorrhea with itching and smarting pain may be among the first symptoms. Cultures of the secretions usually show white staphylococci and other organisms of the normal conjunctiva.

The diagnosis is made by skin tests; biologic treatment consists in desensitization by subcutaneous injections of extracts of the pollen or pollens responsible for the hay-fever. These methods have been previously described.

¹ Ann. Ophthal., 1912, 21, 669.

² Boston Med. and Surg. Jour., 1914, clxxi, 621.

³ Brit. Med. Jour., March 16 and 23, 1912.

Vernal catarrh may have a similar etiology and has been previously discussed.

Treatment of Conjunctivitis.—In gonococcus, streptococcus, pneumococcus, and Morax-Axenfeld conjunctivitis vaccines have proved of aid in treatment according to the reports of Bryan, Medalia, Rubrecht,¹ and others. Their administration would appear to be of particular value in the chronic infections and should be autogenous. In gonococcus conjunctivitis a stock vaccine is usually employed owing to the difficulty of securing the gonococcus in culture.

The vaccine may contain 1,000,000,000 per cubic centimeter and treatment begun with 0.1 c.c. (100,000,000). Subsequent doses are given at intervals of five to seven days in increasing amounts.

Intramuscular injections of 5 to 10 c.c. of milk, boiled for ten minutes and cooled, have been extensively used by European ophthalmologists, and especially by v. Pflugh,² Heineman and Wilke,³ Jendralski,⁴ Uddgren,⁵ and Jickeli.⁶ Berneaud⁷ observed particularly good results in gonorrheal conjunctivitis. Liebermann⁸ found that in gonorrheal infections the milk injections were followed by the lessening of the secretion after the first or second dose, with marked reduction of numbers of gonococci. The primary effect was a chemosis followed by rapid subsidence of inflammation. Corneal ulcers were prevented or arrested if already present.

Klingmüller⁹ has employed subcutaneous injections of a few drops of 20 per cent. solutions of *turpentine* in sterile oil.

In *trachoma*, Friedländer,¹⁰ Hühn,¹¹ Rosenstein,¹² Berneaud, Jendralski, and others have reported beneficial results from intramuscular injections of milk; the reports are so favorable that it would appear that this therapy (5 to 10 c.c. of milk boiled for ten minutes and cooled) is worthy of trial not only in trachoma, but in *follicular* conjunctivitis as well.

Treatment of Keratitis.—Autogenous vaccines may prove of some aid in the treatment of corneal ulcers, as reported by Mayou, Medalia, Rubre, and others; De Schweinitz¹³ has reported the successful treatment of a case of streptococcus hypopyon ulcer with autogenous vaccine; Medalia has reported the successful treatment of 12 cases. These infections are usually pneumococcic or streptococcic; staphylococcic and Bacillus xerosis are frequently secondary organisms. Ulceration of the cornea may also occur as a complication of conjunctivitis caused by the gonococcus and other organisms. Owing to avascular conditions healing is a slow process; if vaccines are used, the usual local measures should not be neglected.

In the treatment of parenchymatous or intestinal keratitis as well as in other forms, intramuscular injections of 5 to 10 c.c. of *milk*, boiled for ten minutes and cooled, have been used with marked success by Müller and Thanner,¹⁴ Jacovides, Pflugh, Jendralski, Uddgren, Jickeli, and others. Even in syphilitic keratitis it is believed that milk injections are of value in conjunction with antiluetic treatment. Darrier¹⁵ has used intramuscular

¹ Bull. d. l. Soc. Belge d'Ophthal., 1909, 27, 82.

² Wchn. f. Hyg. in Therap. d. Anges, 1917, No. 41.

³ Münch. med. Wchn., 1921, 68, 143.

⁴ Berl. klin. Wchn., 1921, 58, 113.

⁵ Milchinjection in der Ophthalmologie, Stockholm, 1918.

⁶ Bull. of the Ophth. Soc., Egypt, 1919, 80.

⁷ Münch. med. Wchn., 1919, 66, 1040; Klin. Monatsch. f. Augenh., 1918, 61, 303; Berl. klin. Wchn., 1919, 56, 887.

⁸ Wien. med. Wchn., 1918, 68, No. 27.

⁹ Berl. klin. Wchn., 1918, 55, 896.

¹⁰ Wien. klin. Wchn., 1916, 29, 1329.

¹¹ Med. Klinik, 1916, 12, 1120.

¹² Med. Klinik, 1917, 13, 185.

¹³ Therap. Gaz., October 15, 1910.

¹⁴ Ztschr. f. Augenh., 1917, 36, 305.

¹⁵ Clin. Ophth., 1919, 23, 559.

injections of milk and horse-serum (usually diphtheria antitoxin) by mouth, the latter being a favored form of therapy among French clinicians. Jendralski has cautioned against milk injections if perforation of the cornea is threatened. In tuberculous keratitis subcutaneous injections of tuberculin (see next chapter) and intramuscular injections of milk have yielded particularly good results in the hands of several investigators. Possek¹ and Haab² have employed subcutaneous injections of typhoid vaccine, the former using a phenol-killed preparation in dose of 500,000,000 repeated the following day. Particularly good results were reported in luetic (acquired and congenital) infections.

Treatment of Iritis.—Autogenous vaccines have not been commonly employed in the treatment of iritis because cultures cannot be made unless iridectomy is conducted for the relief of glaucoma or for some other purpose. Autogenous vaccines have been made, however, of streptococci, pneumococci, and other organisms from the tonsils and apices of teeth in cases of suspected focal infection responsible for iritis. Stock vaccines of streptococci have been given in cases of rheumatic iritis and are said to give relief of pain and objective improvement in some cases; also stock vaccines in cases of suspected gonococcus iritis. Vaccine therapy has proved helpful in some cases as reported by Reber³ and others; if due to focal infection, care must be exercised in the search and treatment of the primary foci of infection.

Injections are given subcutaneously, the first dose being 100,000,000 or 0.1 c.c. of a vaccine containing 1,000,000,000 per cubic centimeter. Subsequent injections are given at intervals of five to seven days in gradually increasing amounts.

From the standpoint of biologic therapy most benefit follows the intramuscular injection of 5 to 10 c.c. of *milk*, boiled for ten minutes and cooled, as first reported by Müller and Thanner.⁴ As a general rule several injections are required at intervals of five or more days. Igersheimer,⁵ Kraupe,⁶ Veach,⁷ v. Pflugh, Bernaud, Jendralski, Uddgren, Darrier, Jickeli, and others have rendered favorable reports on this method of treatment and it appears well worthy of trial especially in chronic cases. In syphilitic iritis antiluetic treatment should be given and is usually sufficient; in tuberculous infections biologic therapy consists of the injection of both tuberculin and milk.

Vaccine Treatment of Infected Perforating Wounds of the Cornea and Infections Following Cataract Extractions.—Maddox,⁸ Mayou, Bryan, Madalia, and others have reported that autogenous vaccines have proved helpful in the treatment of infected wounds of the cornea due to perforating injuries and cataract operations. Cultures should be made on blood-agar; staphylococci, streptococci, and pneumococci are the usual agents either alone or in combination. The vaccine may contain 1,000,000,000 per cubic centimeter and the first dose 0.1 c.c. by subcutaneous injection. Subsequent injections may be at intervals of five to seven days in gradually increasing amounts. Medalia believes that in low-grade conjunctival infections preliminary injections of autogenous vaccine may be worth while preliminary to cataract operations or simple iridectomy. For this purpose the vaccine may be prepared in the same strength and given in four doses at intervals of three to four days in doses of 0.1, 0.2, 0.4, and 1 c.c., operation being done about seven to ten days after the last injection.

¹ Wien. klin. Wchn., 1919, 32, 753.

² Münch. med. Wchn., 1918, 65, 24.

³ Ophth. Rec., 1916, 25, 225.

⁴ Ophth. Rec., 1916, 25, 225.

⁵ Therap. Halbmonatsh., 1921, 35, 104.

⁶ Ztschr. f. Augenh., 1919, xlii, 105.

⁷ Amer. Jour. Ophth., 1920, 3, 93.

⁸ Ophthalmoscope, June, 1908.

Treatment of Iridocyclitis; Choroiditis; Scleritis; Albuminuric Retinitis.

—Berneaud, Jickeli, Uddgren, Jendralski, and others have reported beneficial effects following the intramuscular injection of milk in the treatment of these affections. Heine¹ treated 17 cases of albuminuric retinitis with injections of 5 to 10 c.c. of milk; vision was improved in 11 cases, whereas in 4 the disease process was retarded. In 2, however, the disease progressed in spite of the injections.

Treatment of Retinal Hemorrhage.—In the treatment of retinal hemorrhage 10 to 20 c.c. of horse-serum is generally injected subcutaneously or intramuscularly. It is better, however, to inject fresh sterile human serum because anaphylactic sensitization and reactions are not produced. In my experience best results have been secured by the intramuscular injection of 20 to 30 c.c. of defibrinated human blood. It is not necessary to type the bloods of donor and recipient unless blood is injected intravenously. The technic of these injections is very simple and is described in Chapter XXXVII. Owing to the stypic action of milk 5 to 10 c.c., boiled for ten minutes and cooled, may be injected intramuscularly, but injections of blood produce less discomfort and better results.

TREATMENT OF HEMORRHAGE AND ANEMIA**Serum and Blood in the Treatment of Melena Neonatorum and Hemophilia.**

—Subcutaneous and intramuscular injections of normal horse-, rabbit-, or human serum have been widely and successfully employed in the treatment of melena and hemophilia; Welch,² Reichard,³ Perkins,⁴ Claybrook,⁵ Franz,⁶ Koch and Klein,⁷ Myers,⁸ Freeman,⁹ and others have reported successful results. In melena Berghausen¹⁰ has injected citrated human blood by way of the superior longitudinal sinus.

The effects of the serum injections are probably due to the alterations in the amount of fibrinogen and thrombokinase that follow injections of various non-specific agents, as reported by Moll,¹¹ Modrakowski, and Orator.¹² Wohlegmut¹³ states that the increase in fibrinogen probably results from liver stimulation, but that the thrombokinase arises elsewhere.

The dose of horse-serum for children is ordinarily 10 to 20 c.c. by subcutaneous or intramuscular injection; for adults 20 to 30 c.c. are ordinarily administered. Subsequent injections are given if hemorrhage continues or commences at a later period.

Human serum is preferable to horse-serum because anaphylactic sensitization does not occur. The serum is collected by securing blood from a healthy adult, allowing it to coagulate, and separating the serum. The technic is described in Chapter XXXVII. It is not necessary to type the bloods of donor and recipient if the injections are given subcutaneously or intramuscularly. The doses are the same as given above.

¹ Münch. med. Wchn., 1920, 57, 1221.

² Amer. Jour. Med. Sci., 1910, 139, 800.

³ Jour. Amer. Med. Assoc., 1912, 59, 1539.

⁴ Jour. Amer. Med. Assoc., 1912, 59, 1539.

⁵ Jour. Amer. Med. Assoc., 1912, 59, 1540.

⁶ Münch. med. Wchn., 1913, 59, 2905.

⁷ Gynäk. Rundsch., 1912, 6, 597.

⁸ Arch. Pediat., 1912, 29, No. 3.

⁹ Amer. Jour. Obstet., 1917, 76, 354.

¹⁰ Jour. Amer. Med. Assoc., 1918, 70, 514.

¹¹ Bert. z. chem. Phys. u. Path., 1904, 4, 578.

¹² Wien. klin. Wchn., 1917, 30, 1093.

¹³ Berl. klin. Wchn., 1917, 54, 87.

Whole human blood is probably best of all because these injections furnish not only serum constituents, but corpuscular elements as well. For subcutaneous and intramuscular injection a 20 c.c. syringeful of blood may be taken from a vein of an adult and before coagulation occurs the syringe is emptied in the gluteal muscles of the patient. Or 20 c.c. of blood may be collected in a sterile flask containing 2 c.c. of 10 per cent. solution of sodium citrate and the mixture injected subcutaneously or intramuscularly. The blood may also be collected in a small sterile flask containing glass beads, whipped about for defibrination, and subsequently injected. These methods are described in more detail in Chapter XXXVII. Blood transfusion has also been employed as described in Chapter XLII.

When a large amount of blood has been lost it may be necessary not only to stop bleeding, but restore blood volume; in this case blood transfusion should be given. In newborn infants the transfusion is readily conducted through the superior longitudinal sinus. Methods are described in Chapter XLII.

Treatment of Hemorrhage in Disease and After Operations.—Hemorrhagic retinitis, intestinal bleeding in typhoid fever and in connection with cirrhosis of the liver, in pulmonary tuberculosis, and in some cases of uterine disease have been successfully treated by subcutaneous or intramuscular injections of horse-serum, human serum, or whole human blood. The indications for this therapy are especially clear when the coagulation time of the blood is below normal. Intramuscular injections of citrated or defibrinated human blood are especially efficacious; the injections should be repeated at intervals of four to six hours until bleeding is checked. Blood transfusion has also been employed (see Chapter XLIII).

Kronheimer¹ has used subcutaneous injections of human serum in dose of 5 c.c. for parenchymatous hemorrhage in large septic wounds; Vonchen² has employed injections of whole human blood in the successful treatment of a case of persistent bleeding after liver injury. Meyer³ has employed subcutaneous injections of human serum in the treatment of hemorrhage of icteric patients after operations; Thiesen⁴ has used injections of serum as a preventive of hemorrhage in nose and throat operations when the coagulation time has been found below normal. Barringer⁵ has reported the successful treatment by injections of fresh normal human serum of unilateral kidney hemorrhage in a hemophiliac, and advises that this simple treatment should be tried in similar cases of varicose veins of the renal papilla. Levison⁶ has reported the successful checking of hemorrhage from the urinary bladder following a simple operation by performing cystostomy, removing clots, and filling the bladder with sterile horse-serum.

For the prevention of hemorrhage in operations upon icteric individuals or those having a lowered coagulation time of the blood for any reason, 20 to 30 c.c. of defibrinated human blood may be injected into the gluteal muscles about three hours before operation. Or the blood may be held in readiness and the injection given after the operation if bleeding occurs. A second injection should be given within four to six hours if bleeding is not checked.

Treatment of Purpura Hæmorrhagica.—Subcutaneous and intramuscular injections of horse- or human sera have been used successfully in the

¹ Münch. med. Wchn., 1914, 62, No. 1.

² Jour. Amer. Med. Assoc., 1920, 75, 307.

³ Surg., Gyn., and Obst., 1912, 13, 152.

⁴ New York Med. Jour., 1914, 100, 849.

⁵ Jour. Amer. Med. Assoc., 1912, 59, 1538.

⁶ Jour. Amer. Med. Assoc., 1913, 60, 721.

treatment of purpura, as reported by Class,¹ Moss,² Bodenheimer,³ and others. Whole human blood has been used by Emsheimer,⁴ Cohen,⁵ and others.

Human serum and defibrinated human blood by intramuscular injection are to be preferred. For adults the dose may be 20 to 30 c.c. and repeated if necessary. Blood transfusion has also been employed (see Chapter XLII).

Döllken⁶ assumes that bleeding in purpura is largely due to increased fragility of the blood-vessels and alteration in the coagulability of the blood. He has employed intramuscular injections of 5 c.c. of milk, boiled for ten minutes and cooled, every three days, with very successful results. Bleeding into the tissues usually stopped within five hours after injection and all cases having albuminuria before hand showed clear, albumin-free urine after the injections. A moderate leukocytosis was observed, but no changes in the platelets.

Treatment of Anemia.—Severe secondary anemia as a result of hemorrhage following injury or operation may be treated with the transfusion of blood. Secondary anemia due to repeated losses of small amounts of blood may be benefited by intramuscular or subcutaneous injections of normal human serum or defibrinated human blood; Müller⁷ has employed intramuscular injections of 5 to 10 c.c. of milk, boiled for ten minutes and cooled, because of its well-known styptic effects. Of course most attention should be given the treatment of the cause for bleeding or blood destruction if it can be found and reached therapeutically.

In pernicious anemia blood transfusion usually gives temporary relief and must be repeated at intervals, as discussed in more detail in Chapter XLII. Grote⁸ has employed intramuscular injections of 5 to 10 c.c. of milk, with temporary improvement.

The etiology of the disease is unknown, although very probably a microbic infection. Until the cause is discovered specific serum therapy is not possible, although the subcutaneous or intravenous injection of horse-serum will probably bring about temporary improvement by non-specific agencies as has been found for milk and vegetable proteins.

TREATMENT OF ACUTE ANTERIOR POLIOMYELITIS

Infection and Immunity in Acute Anterior Poliomyelitis.—Studies conducted during the great epidemic of this disease in 1916 have added considerably to our knowledge of its cause and immunity.

The specific virus is filtrable and infection probably occurs through the upper respiratory tract, although Flexner and his associates have succeeded in infecting monkeys not only by application of the virus to the nasal mucous membrane, but by intracerebral, intravenous, intraperitoneal and subcutaneous injection, and by the oral administration of large doses as well.

The first successful transmission of the disease was in 1909 by Landsteiner and Popper,⁹ who succeeded in transmitting the disease to monkeys by inoculating them, intraperitoneally, with the spinal cord of a child who died of poliomyelitis, but they did not succeed in transmitting the infection from monkey to monkey, probably because they used too mild a case. Later, in 1909, Flexner and Lewis¹⁰ obtained the same result and further

¹ Arch. Int. Med., 1906, 6, 170.

² John Hopkins Hosp. Bull., 1911, 22, 272.

³ New York Med. Jour., 1914, 100, 745.

⁴ Jour. Amer. Med. Assoc., 1916, 65, 20.

⁵ Arch. méd. belges, 1920, 73, 988.

⁶ Berl. klin. Wchn., 1919, 56, 226.

⁷ Deutsch. med. Wchn., 1919, 14, 323.

⁸ Münch. med. Wchn., 1919, 66, 307.

⁹ Ztschr. f. Immunitätsf., 1909, 11, 377.

¹⁰ Jour. Amer. Med. Assoc., 1909, 53, 1639, 1913, 2095.

transmitted the infection from monkey to monkey through an indefinite number of passages. Landsteiner and Levaditi¹ in 1909 also transmitted the disease from monkey to monkey and found that the virus remained virulent for some time outside of the body; that the degenerated nerve-cells are taken up by phagocytes, and that there is an analogy between the lesions of poliomyelitis and those produced by rabies. They also demonstrated that the virus is filtrable. Leiner and Weisner² transmitted the infection from monkey to monkey, and found that young animals were more susceptible to infection than older ones, and that the spinal fluid, blood, and spleen were negative. Flexner and Lewis³ transmitted the disease by inoculating very large amounts into the blood or peritoneal cavity, also by the subcutaneous method, and independently found the virus to be filtrable. Landsteiner and Levaditi⁴ found the virus in the salivary glands, and suggested the saliva, moist or dry, as a source of infection. They also found the spinal fluid negative in monkeys dying from artificial infection.

Soon after this, in 1913, Noguchi and Flexner⁵ announced that they had obtained cultures in media similar to the medium used by Noguchi in cultivating spirochetes. In such media in about five days the pieces of tissue employed become surrounded by an opalescent haze which increases for five days more, and a sediment gradually forms. Giemsa's stain shows the presence of minute globoid bodies (0.15 to 0.13 microm. diam.) in pairs, short chains, and masses. Cultures were also obtained from the filtered virus. Monkeys inoculated with these cultures for a variable number of culture generations may die with typical lesions of the disease. The authors consider these bodies the cause of the disease. They further report that the cultures are filtrable through coarse filters.

Earlier bacteriologic studies discovered various micrococci in the tissues of the spinal cord and brain, sometimes in the spinal fluid. Bülow-Hansen and Harbitz⁶ and Harbitz and Scheel,^{7,8} found a diplococcus in the cerebrospinal fluid of several cases of acute anterior poliomyelitis and referred to the work of Geirsvold,⁹ who found a diplococcus in the cerebrospinal fluid of 12 cases and claimed to have produced paralysis and death in experimental animals with them. Pasteur, Foulerton, and McCormac¹⁰ reported the discovery of a micrococcus in the cerebrospinal fluid of a case during life, which produced in rabbits symptoms resembling the disease in human subjects. Leiner and von Wiesner¹¹ and Krause and Meinicke¹² reported that these micrococci were not the etiologic agents of acute poliomyelitis, as had Flexner and Lewis, who first showed that the etiologic agent was filtrable through dense filters and probably belonged to the filtrable viruses¹³; simultaneous and similar results were observed and reported by Landsteiner and Levaditi.¹⁴ Dixon, Fox, and Rucker¹⁵ also found a diplo-

¹ *Compt. rend. Soc. de biol.*, 1909, 62, 592.

² *Wiener klin. Wchn.*, 1909, 22, 1698.

³ *Jour. Amer. Med. Assoc.*, 1909, 53, 1639, 1913, 2095.

⁴ *Compt. rend. Soc. de biol.*, 1909, 67, 787.

⁵ *Jour. Exp. Med.*, 1913, 18, 461.

⁶ *Norsk. Mag. f. Lægevid.*, 1898, 13, 1170.

⁷ *Jour. Amer. Med. Assoc.*, 1908, 1, 281.

⁸ *Jour. Amer. Med. Assoc.*, 1907, 49, 1420.

⁹ *Norsk. Mag. Loegevid.*, 1905, 3, 1280.

¹⁰ *Lancet*, 1908, 1, 484.

¹¹ *Wien. klin. Wchn.*, 1910, 23, 817.

¹² *Deutsch. med. Wchn.*, 1909, 35, 1825.

¹³ *Jour. Amer. Med. Assoc.*, 1909, 43, 2095.

¹⁴ *Compt. rend. Soc. de Biol.*, 1909, 57, 592.

¹⁵ *Rep. Comm. Health. Penn.*, 1907, 420.

coccus in the cerebrospinal fluid, nose, and throat of patients with acute anterior poliomyelitis, and Rucker made a very complete study of this diplococcus which was apparently identical with that recently described by Mathers¹ and Nuzum and Herzog.² While the latter have reported the successful infection of various laboratory animals with these cocci and the reproduction of a disease with clinical symptoms and lesions similar to those of acute poliomyelitis, the experiments of the former were negative throughout; an inoculated monkey succumbed with a hemorrhage meningitis, but without clinical or histologic evidences of anterior poliomyelitis.

Rosenow, Towne, and Wheeler³ recovered what they called a "peculiar polymorphous streptococcus" from the tonsils, brain, cord, mesenteric lymph-glands, and once from the blood, but never from the cerebrospinal fluid of cases of acute poliomyelitis, which produced lesions and symptoms among the lower animals regarded as those of acute poliomyelitis. He has described changes in size and staining reaction of these streptococci according to the culture-medium employed, the age of the culture, and whether they have been grown aerobically or anaerobically. The cocci were said to become very small under anaerobic conditions and to approach in size the globoid bodies described by Flexner and Noguchi; the small forms were found to be filtrable through Berkefeld filters, while the larger forms were not.

There is, therefore, two schools of thought regarding the etiology of epidemic poliomyelitis: one regards the globoid bodies as the primary etiologic agent of the disease and most experimental evidence is in favor of this view. The second regards the streptococci and micrococci found in the tissues as the primary agents.

Brown, Freese, and myself⁴ found streptococci, diplococci, and other organisms not only in the spinal cord and brain of poliomyelitic cases, but in some of the internal organs as well. We were never able to produce the disease experimentally with these cultures. Heist, Cohen, and myself⁵ succeeded in cultivating the globoid bodies and believe them distinct from the aerobic micrococci. The writer is of the opinion that the globoid bodies are the cause of epidemic poliomyelitis and that the disease favors secondary invasion and infection with streptococci, which may possess sufficient virulence to add to pathologic lesions just as streptococci do in scarlet fever.

Whether or not natural immunity to the disease exists is not known; certainly normal human serum cannot neutralize the virus *in vitro* or protect the experimental animal against infection, as does the sera of human convalescents from the disease. Children during the first dentition are the most frequent sufferers, but in times of epidemics adults may become infected. It is highly probable that the majority of children are susceptible, but may escape infection by fortuitous circumstances. Some degree of natural immunity probably develops with adult age, but it is not humoral, that is, neutralizing principles are not present in the blood.

Individuals recovering from the disease are usually immune for life, but second attacks have occurred. The serum of recovered individuals contains immunity principles capable of killing the virus in the test tube under proper conditions and of protecting monkeys against the disease when injected intraspinally. Convalescent serum is, therefore, of value in the treatment of the disease. Complement-fixing antibodies and opsonins have been found in convalescent sera by Heist, Cohen, and myself. Mathers

¹ Jour. Amer. Med. Assoc., 1916, 67, 1019.

² Jour. Amer. Med. Assoc., 1916, 67, 1205.

³ Jour. Amer. Med. Assoc., 1916, 67, 1202.

⁴ Jour. Exper. Med., 1917, 25, 789.

⁵ Jour. Infect. Dis., 1918, 22.

and Tunnicliff,¹ Heist, Cohen, and myself,² Rosenow and Gray³ also found immune opsonins and other specific antibodies in convalescent sera for the streptococci of Rosenow and Mathers; this indicates that these micrococci are not always saprophytic or agonal, but probably possess sufficient virulence to stimulate antibody production and possibly add to the pathologic changes of the disease.

Vaccines in the Treatment of Poliomyelitis.—In Chapter XXXV mention has been made of attempts toward evolving a successful method of prophylactic vaccination along lines similar to vaccination against rabies; vaccines have not been employed in the treatment of the disease of human beings and have generally failed to modify the course of the disease in monkeys.

Treatment of Epidemic Poliomyelitis with Convalescent Serum.—The discovery of the filtrable nature of the micro-organism or virus causing the disease was quickly followed by the observation by Flexner and Lewis⁴ that recovery from an attack of experimental poliomyelitis afforded protection to a second inoculation; and this, in turn, was followed by the detection of immunity or neutralizing substances in the blood-serum, first of recovered monkeys and then of recovered human beings by Levaditi and Landsteiner,⁵ Römer and Joseph,⁶ Flexner and Lewis,⁷ and Anderson and Frost.⁸ Since recovery from an attack of poliomyelitis was obviously brought about through a process of immunization similar to that in other infectious diseases, Flexner and Lewis⁹ endeavored to prevent the development of the infection in inoculated monkeys through the administration of blood-serum taken (*a*) from recovered monkeys and (*b*) from recovered human beings. The results, while not constant and regular, were definite.

These experimental results were at once utilized as a basis of a serum therapy in man by Netter and his associates,¹⁰ who have reported a total of 34 cases of acute poliomyelitis which they have treated by the subdural method of injecting immune serum. They have undoubtedly established the fact that, as in the monkey, subdural injections intelligently carried out in man are safe. They believe, further, that they have proved them to be definitely beneficial or curative. They became increasingly convinced that the period of the disease at which the injections were made counted vitally, and they urged that the injections should be made as early as possible in the course of the infection. Further reports by Sophian,¹¹ Alfaro and Hitce,¹² Wells,¹³ Le Boutillier,¹⁴ Petty,¹⁵ Zingher,¹⁶ Draper,¹⁷ Acuna,¹⁸ and Amoss and Chesney¹⁹ indicate that serum taken from recently recovered

¹ Jour. Amer. Med. Assoc., 1916, 67, 1935.

² New York Med. Jour., September 1, 1917.

³ Jour. Infect. Dis., 1918, 22, 345.

⁴ Jour. Amer. Med. Assoc., 1910, liv, 45.

⁵ Compt. rend. Soc. de biol., 1910, lxxviii, 311.

⁶ Münch. med. Woch., 1910, lvii, 568.

⁷ Jour. Amer. Med. Assoc., 1910, liv, 1790.

⁸ Jour. Amer. Med. Assoc., 1911, lvi, 663.

⁹ Jour. Amer. Med. Assoc., 1910, liv, 1780.

¹⁰ Compt. rend. Soc. de biol., 1911, lxx, 625; Bull. Acad. méd., 1915, lxxiv, series 3, 403; Bull. et mém. Soc. méd. hôp., Paris, 1916, xl, series 3, 299.

¹¹ Jour. Amer. Med. Assoc., 1916, lxxvii, 426.

¹² Semaine méd., 1915, xxii, 211.

¹³ Jour. Amer. Med. Assoc., 1916, lxxvii, 1211.

¹⁴ Amer. Jour. Med. Sci., 1917, 153, 188.

¹⁵ New York Med. Jour., December 16, 1916.

¹⁶ Jour. Amer. Med. Assoc., 1917, 68, 817.

¹⁷ Jour. Amer. Med. Assoc., 1917, 68, 1153.

¹⁸ Arch. Latino-Amer. d. Ped., 1918, 12, 1.

¹⁹ Jour. Exper. Med., 1917, xxv, 581.

cases of poliomyelitis may be employed in its treatment and probably yields the best results. The earlier in the course of the disease the serum is employed in suitable doses, the more promise there is of benefit. The action of the serum appears to be more precise and definite in arresting paralysis than in rapidly bringing about its retrogression.

Physicians have usually administered the serum by intraspinal injection, but the experiments of Flexner and Amoss¹ ascertained that an aseptic meningitis set up by an intraspinal injection of any serum permits the passage of immunity principles from the blood into the cerebrospinal fluid. Therefore the intravenous injection of serum may influence the course of the disease. In the serum treatment of epidemic poliomyelitis Amoss and Chesney state that the following conditions should be observed: (1) Early and prompt diagnosis and treatment; (2) intraspinal injection of immune serum; (3) intravenous or intramuscular injection of immune serum; (4) the serum employed should be collected from cases which have recently passed through an attack of poliomyelitis, as it is to be supposed that the serum will contain a greater amount of immune principles in this early period than after the lapse of many years. The use of serum from recently recovered persons otherwise healthy involves no risk in transferring the micro-organism of poliomyelitis, as the virus has never been detected in the circulating blood of human beings even in the first days of the disease.

Blood should be collected under aseptic precautions and the serum separated, submitted to the Wassermann test, cultured for sterility, and kept in a cold place, preferably without the addition of a preservative. From an adult in good condition ordinarily 500 to 700 c.c. of blood may be removed; from children of twelve years about 200 to 250 c.c. The addition of 0.2 per cent. tricresol does not impair the curative power. In children 10 to 20 c.c. of serum may be injected intraspinally, and 40 to 80 c.c. intramuscularly or intravenously. Adults should receive larger doses. If given before the onset of paralysis one injection may be sufficient; in cases in which some degree of paralysis develops soon after the injection, reinjection twelve to twenty-four hours later may be advantageous.

Treatment of Epidemic Poliomyelitis with Other Sera.—The disease has been also treated with intraspinal injections of normal human serum and normal horse-serum (or horse-serum diphtheria antitoxin). Some physicians believed that good results were obtained, but the New York Department of Health² observed negative results, and Flexner and Amoss have found that these sera not only failed to protect monkeys against the disease, but actually increased their susceptibility to infection by favoring the passage of the virus from the blood to the tissues of the spinal cord. These sera should not be employed in treatment.

Rosenow³ and Nuzum and Willy⁴ have prepared immune sera by the immunization of horses with the streptococci and other micrococci from fatal cases of poliomyelitis. Rosenow believes that the intraspinal injection of this serum reduced the mortality of the disease from about 35 to approximately 6 per cent. Nuzum believed that treatment with his serum reduced the mortality from about 32 to 7.5 per cent. Nuzum found that his immune serum neutralized the poliomyelitic virus, but Amoss and Eberson⁵ were

¹ Jour. Exper. Med., 1914, xx, 249; *ibid.*, 1917, xxv, 449.

² Monograph on Poliomyelitis, 1917, 245.

³ Jour. Amer. Med. Assoc., 1917, 69, 261; *ibid.*, 1917, 69, 1074; Jour. Infect. Dis., 1918, 22, 379.

⁴ Jour. Infect. Dis., 1918, 22, 301, 258; Jour. Amer. Med. Assoc., 1917, 69, 1247.

⁵ Jour. Exp. Med., 1918, 28, 323.

unable to confirm these results and believe that it is inadvisable to employ these sera in the treatment of the disease.

Tsen¹ has endeavored to produce an immune serum in rabbits by immunization with poliomyelitic virus, but with negative results. Up to the present time an immune serum for the virus prepared by the immunization of horses with emulsions of poliomyelitic brain and spinal cord or cultures of the globoid bodies, has not been successfully accomplished.

In so far as the serum treatment of epidemic poliomyelitis is concerned, it would appear that the proper procedure is the intraspinal injection of 10 to 30 c.c. of human convalescent serum and the intravenous injection of 40 to 100 c.c. of the same serum, as soon as possible. After these sera have been administered Rosenow serum may be injected intraspinally in dose of 10 to 30 c.c. for the treatment of the secondary streptococcus infection. In the absence of human convalescent serum, the antistreptococcus serum alone may be employed, but normal human and normal horse-sera should not be administered.

TREATMENT OF LETHARGIC ENCEPHALITIS

Since lethargic encephalitis was first described by Von Economo² opinions have varied in regard to its relation to epidemic poliomyelitis. Strauss, Hirshfeld and Loewe³ have demonstrated that the disease is due to a filtrable virus, and Loewe and Strauss⁴ have succeeded in cultivating a small anaërobic coccus resembling the globoid bodies of poliomyelitis. These investigators regard the disease as etiologically and clinically distinct from poliomyelitis. Amoss⁵ found that the sera of recently recovered human cases of encephalitis were unable to neutralize the virus of poliomyelitis—and regards the two diseases as separate entities on the basis of these and other laboratory experiments and clinical findings; Neustaedter, Larkin and Banzhaf,⁶ however, report opposite results, that is, that monkeys were protected against poliomyelitis by the administration of sera from human convalescent cases of encephalitis. The subject, therefore, is undecided.

Grunwald⁷ has injected 80 to 100 c.c. of convalescent serum intraglutely in the treatment of the disease, with apparently some success. If sufficient serum is available it would appear advisable to administer it intraspinally and intravenously in the same manner as described for the treatment of poliomyelitis.

Brill⁸ reports the successful treatment of several cases by the intraspinal injection of 25 to 35 c.c. of the patient's own unheated serum.

Laubie⁹ reports the rapid recovery of patients following intraspinal injection of tetanus antitoxin, but this is virtually normal horse-serum in so far as encephalitis is concerned, and the method cannot be endorsed unless normal horse-serum is shown to possess neutralizing principles for the virus, which so far has not been accomplished.

TREATMENT OF MALTA FEVER

Immunity in Malta Fever.—Malta fever or undulant fever is a typhoid-like infection caused by the *Micrococcus melitensis*. The disease is found in

¹ Jour. Immunology, 1918, 3, 213.

² Wien. klin. Wchn., 1917, 30, 581.

³ New York Med. Jour., 1919, cix, 772; Jour. Infect. Dis., 1919, 25, 378.

⁴ Jour. Amer. Med. Assoc., 1919, 73, 1056; Proc. New York Path. Soc., 1920, 20, 18.

⁵ Jour. Exper. Med., 1921, 33, 187.

⁶ Amer. Jour. Med. Sci., 1921, 162, 715.

⁷ Deutsch. med. Wchn., 1920, xlv, 45.

⁸ Med. Record, 1920, 97, 1079.

⁹ Bull. de l'Acad. de méd., 1920, 83, 246.

the countries of the Mediterranean basin, India, China, Philippine Islands, and in some parts of America (Texas).

The majority of human beings are probably susceptible, and antibodies (opsonins and lysins) are not ordinarily found in normal sera. The majority of the lower animals are naturally immune. Monkeys, however, are quite susceptible, and may be infected by subcutaneous and oral administration of the organisms; guinea-pigs and rabbits are less easily infected. Goats suffer with the disease and pass the organisms in the milk, urine, and feces; or their milk becomes contaminated by feces and constitutes an important means of spread of the disease. The organism exhibits a peculiar affinity for the spleen, lymph-nodes, liver, and sex organs, and may persist in these tissues for two months. By some bacteriologists the organism is regarded as a bacillus rather than a coccus, and Meyer, Shaw, and Fleischner¹ have recently shown that the lesions produced in the guinea-pig are quite similar to those produced experimentally by *Bacillus abortus*.

During an attack of Malta fever in both man and the lower animals, agglutinins, opsonins, bacteriolysins, and complement-fixing antibodies are produced and are useful in the serologic diagnosis of the disease.

The immunity is very similar to the processes concerned in the resistance and immunity of typhoid fever. One attack usually protects for the balance of life, but second and even third attacks have been known to occur.

Vaccine and Serum Treatment.—Vaccines of *Micrococcus* or *Bacillus melitensis* have been used by Eyre² for prophylactic purposes, but only on a small scale and with inconclusive results. A serum prepared by the immunization of horses has also been employed in the treatment of human and experimental monkey infections, but with indifferent results.

Owen and Newham³ have reported beneficial effects from the treatment of the disease with a vaccine.

Owing to the usual mildness of the infection with a mortality of only 2 per cent., specific biologic therapy has not been extensively employed, but I surmise that results similar to those observed in typhoid fever may be obtained from the employment of vaccines for prophylactic and curative purposes.

TREATMENT OF TUMORS

Immunity in Tumors in Relation to Biologic Therapy.—One result of the discovery of spontaneous malignant tumors in mice, rats, and other lower animals and their transmissibility to normal animals of the same species by injecting bits of tumor tissue has been a very large amount of investigation on the subjects of natural and acquired immunity to these growths. Just how much of the knowledge gained by these investigations is applicable to tumors of human beings, and especially carcinomata and sarcomata, is difficult to state, but it is highly probable that the conditions initiating and governing neoplasms among the lower animals are similar to those operative among human beings.

Examples of natural immunity to neoplasms are frequent and striking. Some mice cannot be inoculated with a tumor easily transmissible to other mice of the same species; mouse tumors may not be transmitted to rats, and the reverse. Tumors of one species of mice may not be transmitted to mice of a different species. A mouse immune to one transmissible tumor may be susceptible to another tumor. Similar transmission or inoculation experiments, of course, cannot be made among human beings, but it is entirely

¹ Jour. Infect. Dis., 1922, 31, 159.

² Kolle and Wassermann's Handbuch, 1913, 4, 421.

³ Lancet, 1915, 2, 529.

likely that a similar state of affairs exists, and that some individuals would be found with a high natural resistance just as other human beings may apparently inherit a predisposition, as shown by the occurrence of cancer among individuals of the same family stock.

Sex has no bearing upon natural immunity to neoplasms among the lower animals; among human beings, however, sex does have some influence, in so far as the incidence of carcinoma is concerned, being more frequent among women than men. *Age* is an important factor among human beings, carcinoma being most frequent after thirty years of age, although sarcoma and benign growths may occur at any age. Among the lower animals, however, age has little or no influence; Loeb,¹ Bashford and Murray,² Buschke,³ Ehrlich and Apolant,⁴ and others found mouse tumors transmissible to both young and old animals, although half-grown animals are probably best for experimental work. It is probable that the influence of age of human beings upon the incidence of cancer is more a matter of influencing etiologic factors than actual natural resistance.

As previously stated, *race* has a great influence upon the transmission of tumors among the lower animals; whether a similar state of affairs exists among human beings cannot be stated. Neoplasms occur, however, among all races of human beings.

Pregnancy exerts a restraining influence upon tumor growths in the lower animals according to Haaland,⁵ Uhlenhuth and Weidanz,⁶ and others; Herzog,⁷ on the other hand, thought that pregnancy enhanced tumor growth among mice. Fichera⁸ explained these discrepancies with the statement that when several embryos were present, specific food-stuffs were absorbed by them, leaving but little for the tumor, whereas when few embryos were present, sufficient specific pabulum remained for the nourishment of the tumor. In women, however, pregnancy does not appear to affect malignant tumors in a constant manner, although fibromyomata of the uterus are known to have remained stationary in size and even to have retrogressed during this state.

Tyzzer⁹ found that the mother mouse could transmit her natural resistance to a neoplasm to her young; Clowes,¹⁰ however, had previously shown that the young of resistant mothers could be inoculated. Susceptibility to inoculation, however, is surely transmissible, as shown by Moran,¹¹ Cuénot and Mercier,¹² and others. As will be shortly discussed, it is possible to produce active immunity against neoplasms in mice, but this induced or artificial immunity is not transmissible, as shown by Bashford, Murray, and Haalnad.¹³

Of even greater interest, and especially from the standpoint of biologic therapy, is the peculiar form of active immunity that may be induced against malignant tumors among mice and rats. Clowes and Baeslack,¹⁴

¹ Jour. Med. Res., 1901, N. S., 1, 36.

² Sci. Reports, Cancer Research Fund, London, 1904, 1, 14.

³ Berl. klin. Wchn., 1911, 48, 215.

⁴ Berl. klin. Wchn., 1905, 42, 872.

⁵ Berl. klin. Wchn., 1907, 44, 718.

⁶ Arb. a. d. k. Gesundh., 1909, 30, 440.

⁷ Jour. Med. Res., 1902, 3, 76.

⁸ Quoted by Apolant, Jour. Exper. Med., 1911, 14, 320.

⁹ Jour. Med. Res., 1909, 16, 519.

¹⁰ Johns Hopkins Hosp. Bull., 1905, 16, 130.

¹¹ Arch. d. Méd. exp. e. d'Anat. path., 1894, 6, 692.

¹² Compt. rend. d. l'Acad. d. Sci., 1910, cl, 1443.

¹³ Third Sci. Report, Imperial Cancer Fund, 1908, 395.

¹⁴ Med. News, 1905, 77, 969.

Flexner and Jobling,¹ Lewin,² and others noted that when tumors underwent spontaneous absorption these mice were rendered refractory to reinoculation. Further investigations showed that in a general way the subcutaneous injection of bits of tumor tissue, failing to infect mice the first time, may actually render them more resistant than ever; that is, increase their resistance by a process of active immunization. Ehrlich³ found that this active immunity was not specific; that mice inoculated with cancer tissue were rendered refractory to sarcomata and the reverse. Bashford⁴ had previously recorded that mice inoculated with one strain of cancer tissue were rendered refractory to inoculation with a second and different strain, and he and his associates⁵ later discovered that this immunity was not specific and could be engendered by the injection of normal defibrinated mouse blood. Numerous investigators later confirmed these findings and showed that immunity against inoculable tumors could be engendered in mice by preliminary injections of normal mouse spleen, liver, and other tissues.

Woglom⁶ found injections of sterile chicken embryo skin particularly efficacious, but injections in amounts from 1 to 10 c.c. failed to immunize chickens against inoculation with the Rous chicken sarcoma. Fleisher and Loeb⁷ found that mice with spontaneous tumors were slightly more refractory to transplantable tumors than normal mice; that its own tumor could be transplanted to another part of its body successfully, but rarely to other mice. These investigators found that mice with spontaneously growing tumors were not immunized thereby to the same extent as the immunity engendered by transplantable tumors.

While the immunity is non-specific in the sense that it may be engendered against tumors in general by preliminary injections of normal mouse tissues, it was shown that these tissues must be from mice in order to immunize mice, from the rat to immunize rats, etc. The injection of normal tissues of the rat do not immunize mice, and the reverse. Michaelis⁸ found that the injection of mice with an emulsion of rat carcinoma did not confer an immunity against mouse carcinoma, and the reverse; Lewin,⁹ however, thought that this was possible, but numerous additional investigations have established fairly well that active resistance against tumors is engendered only by intact tumor cells of the same species.

This active immunity has been shown to be general by Hashford, Murray and Cramer¹⁰; that is, subcutaneous injections of tumor cells immunize against subsequent implants by the intraperitoneal and other routes. Woglom¹¹ showed that mice made refractory by subcutaneous injections of embryo skin were resistant to intrarenal as well as to subcutaneous grafts of cancer.

Woglom¹² and others have also shown that this active immunity cannot be engendered by a mouse's own tissues; that is, if a mouse is injected with an emulsion of its own spleen it is not rendered resistant, although this state may be induced by injections of the spleen of another mouse.

Furthermore, Clowes,¹³ Bridé,¹⁴ and others have shown that living cells are necessary; vaccines of heat and chemically killed cancer cells and of the nucleoproteins, do not suffice.

¹ Proc. Soc. Exp. Biol. and Med., 1907-08, 5, 17. ² Ztschr. f. Krebsf., 1907-08, 6, 306

³ Ztschr. f. ärztl. Fortbildung, 1906, 3, 211.

⁴ Brit. Med. Jour., 1906, 2, 209.

⁵ Brit. Med. Jour., 1906, 2, 209; Lancet, 1906, 2, 315; Third Scientific Report, Imperial Cancer Research, 1908, 333, 369.

⁶ Jour. Exper. Med., 1915, 22, 154.

⁷ Jour. Med. Res., 1916, 34, 1.

⁸ Ztschr. f. Krebsf., 1907, 5, 192.

⁹ Berl. klin. Wchn., 1907, 44, 1606.

¹⁰ Proc. Roy. Soc., Series B, 1907, 69, 177.

¹¹ Lancet, 1911, 2, 92.

¹² Ztschr. f. Immunitätsf., 1911, 11, 683.

¹³ Brit. Med. Jour., 1906, 2, 1550.

¹⁴ Ann. de l'Inst. Pasteur, 1907, 21, 768.

Of great importance from the standpoint of prevention of metastases is the question whether a spontaneously growing tumor in a mouse immunizes the animal against reinoculation with bits of the same tumor. Haaland¹ found that this did not occur; furthermore, that the spontaneous tumor did not immunize against other transplantable growths. In human beings there is no adequate immunity reaction to prevent metastases and, more unfortunately, no adequate means at hand for bringing about a resistance. Whether or not the existence of one tumor in a human being immunizes against the development of other tumors of a different kind is doubtful, and probably does not occur. Certainly it is not infrequent to find carcinoma of the cervix and fibromyomata of the uterine body growing simultaneously, and hypernephroma of the kidney has progressed at the same time as a carcinoma of the stomach, etc.

Evidences of the refractory state engendered by injections of living tumor or other cells first appears, according to Ehrlich,² in from seven to fourteen days, and lasts weeks and even months. Woglom³ found that the immunity engendered by injections of embryo skin reached its maximum about the tenth day, remained at a high level until the twenty-fourth day, and then declined, to vanish at about the seventy-fifth day.

Of further interest, and especially from the standpoint of biologic therapy, are the investigations bearing upon the possibility of producing passive immunity to transplantable tumors. Will the blood of a natural resistant mouse protect a susceptible mouse? Is it possible to immunize a rabbit with cancer cells and find that its blood will protect animals against infection with this cancer? Will the blood of mice or other animals rendered refractory or immune by injections of living cancer or normal tissues protect susceptible animals against implantations of tumors? Considerable work has been done on these questions by Clowes and Baeslack,⁴ v. Dungern,⁵ Apolant,⁶ Haaland,⁷ Russel,⁸ and others, with generally negative results, although Gaylord states that passive immunity to cancer does actually exist, even though it is not easily or always demonstrable.

It will be seen, therefore, that malignant tumors of the lower animals and presumably of human beings behave in some respects like microbic infections, in that they are inoculable and may engender a state of active immunity. That this active immunity is not specific is in line with the possibility of engendering active immunity against bacterial infections with non-specific agents; cancer immunity is peculiar, however, in that it may be induced by the injection of cancer or normal cells of the same species (homologous immunization), and requires a vaccine of living cells. Failure of passive immunization against tumors is not peculiar because passive immunization may fail frequently and readily enough in various microbic infections.

What is the nature or mechanism of natural immunity to neoplasms displayed by the lower animals? What is the nature of the artificial or induced immunity engendered by injections of homologous living tumor or normal cells? In mice and rats transplantable tumors may undergo spontaneous disappearance; what mechanism brings about this condition? These are questions that have occupied the attention of numerous investigators

¹ Fourth Sci. Report Imperial Cancer Res., 1911, 79, 83.

² Ztschr. f. ärztl. Fortbildung, 1906, 3, 211.

³ Jour. Exper. Med., 1912, 16, 629.

⁴ Medical News, 1905, 77, 969.

⁵ Ztschr. f. Immunitätsf., 1910, 5, 695.

⁶ Ztschr. f. Krebsf., 1911-12, 11, 106.

⁷ Berl. klin. Wchn., 1907, 44, 717.

⁸ Third Scientific Report Imperial Cancer Research, 1908, 357.

for the past twenty years, and have not yet been answered, although valuable information has been gained.¹

Ehrlich² has offered an explanation resting on the hypothesis that a tumor requires for its growth a certain specific food substance. He found that, by inoculating a virulent and rapidly growing tumor, he could render impossible the growth of a second tumor, and believed that the first tumor had used up the pabulum which creates a condition of *athrepsia* or *athreptic immunity*. Further support for this theory was found in zigzag inoculations; a mouse tumor transplanted to the rat may die owing to a lack of specific pabulum, whereas when transplanted back again to the mouse it lives and thrives, because of the supply of specific food furnished by the latter. Ehrlich has stated that "whenever cancer cells fail to proliferate it means that they fail to obtain the food required either because the normal body cells have a greater avidity for this food or else the cells with which the animal was immunized anchored all the specific food and the cancer cells inoculated subsequently could not obtain it."

This theory has been vigorously attacked by Uhlenhuth and his associates,³ Clowes,⁴ Lewin,⁵ Bridé,⁶ Bashford and Russel,⁷ Levin and Sittenfeld,⁸ and others; on the other hand, Apolant,⁹ Schöne,¹⁰ Borrel,¹¹ and others have just as vigorously defended it. The theory appears acceptable to the writer in so far as offering an explanation of natural resistance; it is possible that these living cells die because of a lack of endocellular nutriment. It may even explain spontaneous retrogression of tumors by exhaustion of a vitamin required for the growth of these cells. It does not explain, however, the mechanism of artificial or induced immunization secured by the injection of vaccines of living cancer or normal tissue cells.

Russel¹² has sought to explain natural and acquired resistance on the basis that the cancer cell when transplanted is unable to excite the connective tissues of the host to produce a "scaffolding" of connective tissue—a failure of vascularization and "specific stroma reaction," which he believes are necessary for successful tumor growth. Woglom¹³ confirmed these observations, but Burgess¹⁴ believed that the resistance of non-susceptible animals was due to an inflammatory lesion interfering with the nutrition of the tumor. Levin¹⁵ believes that the primary factor of resistance is inhibitory action of the cells of the host upon the tumor cells; that the growth of cancer represents a loss of equilibrium between this inhibitory action and the proliferative power of cancer cells, and that the connective tissue reaction is of secondary importance.

Da Fano¹⁶ has stated that the lymphocytes and plasma cells are especially concerned in the mechanism of tumor immunity; this subject has been

¹ For a splendid review of the literature up to 1913 see *Studies in Cancer and Allied Subjects* by Woglom, Columbia University Press, New York, 1913.

² Verhandl. d. deutsch. path. Gesellsch., 1908, 12, 17.

³ Centralbl. f. Bakteriöl., Ref., 1910, 47, 158.

⁴ Brit. Med. Jour., 1906, 2, 1551.

⁵ Berl. klin. Wchn., 1907, 44, 1606.

⁶ Ann. de l'Inst. Pasteur, 1907, 21, 771.

⁷ Proc. Roy. Soc., Series B, 1909, 72, 298.

⁸ Jour. Exper. Med., 1911, 13, 511.

⁹ Ztschr. f. Immunitätsf., 1911, 10, 103; Jour. Exper. Med., 1911, 14, 316.

¹⁰ Deutsch. med. Wchn., 1907, 33, 866.

¹¹ Bull. de l'Inst. Pasteur, 1907, 5, 594.

¹² Proc. Roy. Soc., Series B, 1909-10, 72, 298.

¹³ Fifth Scientific Report Imperial Cancer Research, 1912, 43.

¹⁴ Jour. Med. Res., 1909, 21, 575.

¹⁵ Jour. Exper. Med., 1908, 10, 811; *ibid.*, 1911, 13, 604; *ibid.*, 1912, 15, 163.

¹⁶ Ztschr. f. Immunitätsf., 1910, 5, 1.

especially studied by Murphy¹ in this country, and Murphy and Taylor² have recently summarized the main points of evidence, as follows:

"(1) the accumulation of lymphocytes about a transplanted cancer graft in an immunized animal; (2) the rise in number in the circulating lymphocytes during the development of the immune state, irrespective of whether the type of immunity induced is artificial or natural; (3) the setting aside of the potential immunity by the x -rays where the dosage employed is sufficient to destroy a large part of the circulating lymphocytes; and finally, (4) the abolition of the potential immunity for a special tumor strain by means of the lymphocyte-destroying power of the x -rays. These points are further supported by the observations of Loeb³ on the part played by the lymphocyte in respect to homoplastic grafts of normal tissue, and by those of Murphy⁴ on heteroplastic tissue grafts."

The extensive investigations of Fleischer⁵ on the histologic reactions about transplanted tissues in normal and immunized animals also indicates the importance of the leukocyte reaction.

Failure of passive immunization in tumors of mice suggests that antibodies for tumor cells are not present in the blood, and the majority of investigators support this view. Rous⁶ joined susceptible mice to non-susceptible in parabiosis, but found that nothing developed in the latter to prevent successful transplantation. Kross⁷ has recently failed to confer passive immunity with injections of blood from immune animals, and in parabiotic experiments failed to increase the susceptibility of the immune or decrease the immunity of the susceptible. Tsurum⁸ and others reported the presence of more or less specific complement-fixing antibodies in the sera of immune animals, but the consensus of opinion is to the effect that antibodies are not demonstrable in the blood of animals possessing natural or acquired immunity to transplantable tumors; Lambert and Hanes⁹ succeeded in growing rat sarcoma in immune plasma *in vitro* as successfully as in normal plasma.

Serums, Vaccines, and Autolysates in the Treatment of Malignant Tumors.—Investigations in the immunity of mice and other lower animals to transplantable carcinomata and sarcomata, and especially the states of induced resistance to transplantation resulting from the preliminary injection of emulsions of tumor cells and normal tissues, naturally aroused strong hopes for the development of a form of biologic therapy for the treatment of malignant neoplasms in human beings.

Among the earliest attempts in this field were those by Jensen¹⁰ and v. Leyden and Blumenthal,¹¹ with serums prepared by the immunization of animals with emulsions of tumor cells; Borrel,¹² Bridé,¹³ Lewin,¹⁴ Uhlenhuth,¹⁵ and others, however, have proved that nothing in the way of a specific therapy is to be expected in this direction. Some of these serums undoubtedly contained small amounts of cytotoxins for human tissue cells, but these were without specific effects upon the neoplastic cells of human beings and the lower animals, and too feeble in action to bring about more than the

¹ Jour. Amer. Med. Assoc., 1912, 59, 874; Jour. Exper. Med., 1913, 17, 482; *ibid.*, 1915, 22, 204.

² Jour. Exper. Med., 1918, 28, 1.

³ Jour. Med. Res., 1917, 37, 229.

⁴ Jour. Exper. Med., 1914, 19, 513.

⁵ Jour. Med. Res., 1917, 37, 483; *ibid.*, 1918, 38, 353; *ibid.*, 1918, 39, 1; *ibid.*, 1922, 43, 145.

⁶ Jour. Exper. Med., 1909, 11, 810.

⁷ Jour. Cancer Res., 1921, 6, 25, 121.

⁸ Jour. Path. and Bacteriol., 1915, 20, 214.

⁹ Jour. Exper. Med., 1911, 14, 129, 453.

¹⁰ Centralbl. f. Bakteriöl., 1903, 34, 30.

¹¹ Deutsch. med. Wchn., 1902, 28, 637.

¹² Bull. de l'Inst. Pasteur, 1907, 5, 607.

¹³ Ann. de l'Inst. Pasteur, 1907, 21, 774.

¹⁴ Ztschr. f. Krebsf., 1907-08, 6, 308.

¹⁵ Arb. a. d. k. Gesundh., 1911, 36, 491.

usual degree of spontaneous retrogression. Clowes¹ thought that the serums of mice whose tumors had undergone spontaneous disappearance possessed some curative activity, but Bashford² was unable to confirm these results. In this connection mention may also be made of the work of Freytag,³ who employed injections of blood and serum from mice and other animals in the treatment of mouse cancer with retrogression of the tumors in some, but cures in none; also of the experience of Hodenphyl,⁴ who found that the ascites fluid from a case of human cancer in which the primary and secondary tumors had undergone spontaneous retrogression had some curative influence upon transplantable cancers in mice and in the majority of 47 human cancers. Ill and Minningham⁵ treated 30 additional cases of human cancer with the fluid from a similar case, and while no case was cured observed relief of pain, hemorrhage, and cachexia in a number. Green and Konrad⁶ also observed some improvement of cases of uterine cancer treated with injections of ascites fluid, but believed that the results may have been due to non-specific stimulation; Weil⁷ was unable to determine any difference in the effects of fluids from cancer cases from those of other origin. It is possible that these fluids contained specific anticancer substances, but it is more probable that the temporary beneficial effects were due to non-specific protein reactions.

Vaccines of tumor cells have been used by Blumenthal,⁸ Coca and Gilman,⁹ Coca, Dorrance, and Lebrede,¹⁰ Graff and Ranzi,¹¹ Risley,¹² Vaughan,¹³ and others for the treatment of malignant tumors of the lower animals and human beings; in some instances beneficial results were observed and especially the temporary relief of some of the symptoms, but actual cures and prevention of metastases have not been achieved. Lunckenbein¹⁴ believed that good results were to be obtained by the intravenous injection of a vaccine of the patient's own cancer cells, and more recently Kellock, Chambers, and Russ¹⁵ have expressed the opinion that the subcutaneous injection of these autogenous vaccines of tumor cells may be of value in the prevention of metastases after the primary tumor had been removed surgically, but it is highly probable that the temporary relief of pain, hemorrhage, and cachexia with gain in weight observed among some human cases are to be attributable to the non-specific protein reactions that may be elicited by these substances. The same is probably true of the beneficial effects following the injection of autolysates of tumor cells reported by Bauer, Latzel and Wessely,¹⁶ and Klinger,¹⁷ and of autolysates of human embryos, reported by Fichera¹⁸ and others; likewise of injections of the patient's own blood or serum (auto-serum therapy) reported by Krokiewicz,¹⁹ Paget,²⁰ and others.

¹ Brit. Med. Jour., 1905, 2, 1552.

² Brit. Med. Jour., 1905, 2, 96; *ibid.*, 1906, 2, 209.

³ Ztschr. f. Krebsf., 1910-11, 10, 157.

⁴ Med. Rec., 1910, 67, 359.

⁵ Jour. Amer. Med. Assoc., 1912, 49, 497.

⁶ Boston Med. and Surg. Jour., 1914, 170, 352.

⁷ Jour. Med. Res., 1910, 23, 85.

⁸ Med. Klin., 1910, 6, 1982; Berl. klin. Wchn., 1914, 50.

⁹ Philippine Jour. Sci., 1909, 4, 391.

¹⁰ Ztschr. f. Immunitätsf., 1912, 13, 543.

¹¹ Mitt. a. d. Grenz. d. Med. u. chir., 1912, 25, 211.

¹² Boston Med. and Surg. Jour., 1911, 165; Jour. Amer. Med. Assoc., 1911, 56, 1383.

¹³ Jour. Amer. Med. Assoc., 1914, 63, 1258.

¹⁴ Münch. med. Wchn., 1914, 41.

¹⁵ Lancet, 1922, 1, 217.

¹⁶ Ztschr. f. klin. Med., 1915, 81, 355.

¹⁷ Cor.-Bl. f. schweiz. Aerzte, 1915, 46, 1217.

¹⁸ Bull. de l'Inst. Pasteur, 1911, 9, 272; Lancet, 1911, 2, 1194; Policlinico, July 3, 1910.

¹⁹ Wien. klin. Wchn., 1912, 25, 1311.

²⁰ Med. Record, 1916, 89, 719.

Treatment of Sarcoma with Coley's Fluid.—The old clinical observation that an attack of erysipelas often causes a decrease in the size of malignant tumors, especially sarcomas, received confirmation in the work of Fehleisen, who inoculated tumors with living cultures of streptococci. Among 6 patients so inoculated, a decrease in the size of the tumor was noted in 5. Killed cultures were tried without effect. Coley¹ then tried injections of the toxins of streptococci and later mixtures of the toxins of streptococci (preferably from cases of erysipelas) and *Bacillus prodigiosus*.

Since then *Coley's fluid* has been rather extensively employed in the treatment of sarcomas. There is not a uniformity of opinion in regard to its value, but the majority of surgeons believe that it should be tried in cases of inoperable sarcoma. Coley has always maintained a conservative and scientific attitude and undoubtedly has obtained permanent cures in many cases. In a general way cures have been effected by Coley and other surgeons in from 4 to 9 per cent. of cases. Hunt,² Hill,³ Harmer,⁴ and others have reported favorable results; the latter has summarized the results of 100 inoperable cases proved by microscopic examinations, as follows: "The cases were arranged in six groups, determined by the effect of the toxins. Group A. Cases in which there was no appreciable effect, 12 cases. Group B. Cases in which the growths softened, but did not appreciably diminish in size, 5 cases. Group C. Cases in which growths disappeared or practically disappeared, but returned, 20 cases. Group D. Cases in which growths disappeared, but metastases simultaneously occurred, 10 cases. Group E. Cases in which growths diminished in size, but still persisted, 14 cases. Group F. Apparent cures in which growths disappeared and no metastases have occurred, 73 cases. Spindle-cell sarcoma, 26 cases; 36 per cent. apparent cures. Round-cell sarcoma, 28 cases; 39 per cent. of apparent cures. Melanotic sarcoma, 6 cases, 5 in Group D, one in Group F. Giant-cell sarcoma, 14 cases; 11 apparent cures. Mixed-cell sarcoma, 13 cases; 5 apparent cures."

Harmer concludes that mixed toxins of streptococcus and *Bacillus prodigiosus* are of value in certain cases of inoperable sarcoma. He emphasizes that the treatment of primary or recurrent inoperable sarcoma with mixed toxins must be intensive, and that the institution of this treatment is unjustifiable in cases in which operative measures of reasonable safety offer possible hope for removal. Seventy-three of the cases analyzed after most rigorous criticism have been regarded as apparent cures. The small round-cell type apparently offers the greatest expectation of benefit, followed closely by the spindle-cell type (excluding fibrosarcoma). Only a relatively small number of mixed-cell type have been benefited. The use of toxins in cases with multiple melanotic growths does not seem justifiable, but their use in single melanotic growths is legitimate. Regarding the tissue of origin the greatest number of apparent cures have occurred in bone sarcoma (exclusive of giant-cell cases) over 18 per cent. of the total number of apparent cures, with an equal division of round-cell and small cell types. Giant cell cases furnish about 15 per cent. of the total number of apparent cures. Primary, inoperable, round-cell sarcomata of the cervical glands compose about 10 per cent. of apparent cures. Sarcomata arising from fascia and muscle, which have been apparently cured, have been situated in the lower extremity, abdominal wall, and back. They composed about 16 per cent. of the total number of apparent cures. Nine of 12 are of spindle-

¹ Amer. Jour. Med. Sci., July, 1894; Jour. Amer. Med. Assoc., 1910, 55, 346; Surg., Gyn., and Obstet., August, 1911.

³ Arch. Pediat., September, 1912.

² Lancet, June 17, 1911.

⁴ Boston Med. and Surg. Jour., 1915, 172, 321.

cell type. In a small number of cases the toxins produce striking relief of pain.

The injections are subcutaneous, at first in parts removed from the tumor, and later, if possible, directly into the tumor itself.

TREATMENT OF SYPHILIS

The immunity of syphilis and attempts toward active prophylactic immunization have been discussed in Chapter XXXV; attempts toward passive prophylactic immunization have been reviewed in Chapter XXXVIII.

Vaccine and Serum Treatment.—Nothing definite has yet been accomplished in the treatment of syphilis by the administration of a vaccine of *Spirochæta pallida*; Noguchi's luetin is such a vaccine. As stated in Chapters XXXV and XXXVIII the injection of animals with dead vaccines of *pallida* is followed by very little, if any, antibody production, although I am not sure that the results would be the same in man. It is possible that the subcutaneous injection of a *pallida* vaccine, as luetin, in doses of 0.5 to 1 c. c. may be beneficial, as indicated by the report of Mueller and Planner,¹ who found that the administration of luetin in this way rendered the curative activity of neoarsphenamin more pronounced. My colleague, Dr. Schamberg, and myself, treated a series of "Wassermann fast" cases of syphilis two years ago with a vaccine of *pallida* prepared by cultivating the spirochetes in fluid media, followed by washing, suspension in saline, heating, and careful sterility tests; we found in several an apparent increased susceptibility to arsphenamin with a reduction in the Wassermann reaction, furthermore some curative effect was produced on the lesions by the vaccine alone, so that the subject of vaccine therapy as an adjuvant to the treatment of some cases of syphilis refractory to ordinary measures is one worthy of further study and interest.

As discussed in Chapter XXXVIII, potent immune sera for the prophylaxis and treatment of syphilis have not been prepared by the immunization of the lower animals. Very possibly the sera of human syphilitics in the latent stages possess immune principles, but these sera have not been employed in the treatment of syphilis, although it is possible that some good may be accomplished in both a specific and non-specific way by the intravenous injection of such sera.

Non-specific Treatment.—Just as the subcutaneous injection of *pallida* vaccine (luetin) may aid in the treatment of syphilis by rendering the action of mercury and arsphenamin more effective, so likewise other protein agents may have similar effects.

Uddgren² has observed that the intramuscular injection of milk may provoke a positive Wassermann reaction in syphilitics with previous negative reactions; this effect is probably due to some stimulating action upon foci of the disease. Stückgold,³ Schreiner,⁴ Schacherl,⁵ Hauber,⁶ and others have employed injections of milk, peptone, tuberculin, and other agents as adjuvants to treatment with mercury or arsphenamin, observing a more rapid clinical and serologic improvement in syphilitics, especially tertiary, nerve, and congenital cases.

¹ Berl. klin. Wchn., 1918, lv, 354.

² Berl. klin. Wchn., 1918, lv, 354.

³ Ueber den Einfluss von Interkurrenten fieberhaften Krankheiten und von Fieberzustände durch intragluteale Milchinjectionen hervorgerufen sind auf den Verlauf der Syphilis, mit besonderer Berücksichtigung der Congenitaler, Berlin, 1919.

⁴ Wien. klin. Wchn., 1920, 33, 34.

⁵ Jahr. d. Psych. u. Neurol., 1914-15, 35, 27, 207.

⁶ Ztschr. f. d. Ges. Neurol. u. Psych., 1914, 24, 1.

A large number of non-specific agents have been employed in the treatment of *paresis*, especially fever-producing agents, as tuberculin, and those producing leukocytosis, as nuclein.

v. Jauregg¹ has employed subcutaneous injections of Koch's old tuberculin every two days, beginning with 0.01 gm. and increasing gradually to 0.5 gm. He and Pilez,² Hudovernig,³ Battistessa,⁴ Jukow,⁵ and others have reported that the results were more favorable when the injections were employed along with the administration of mercury and arsphenamin than when the latter alone were used.

Donath,⁶ Fisher,⁷ Szedlák,⁸ and others have employed subcutaneous injections of 0.5 to 3 c.c. of 10 per cent. solutions of nuclein every three to five days for the purpose of producing leukocytosis, and reported that the results were better when employed along with mercury and iodids or arsphenamin than when the latter alone were employed; Brown and Ross,⁹ however, were not able to confirm these results.

v. Jauregg has also employed staphylococcus vaccine; Friedländer has used typhoid vaccine, and Plant a mixed vaccine of staphylococci and streptococci for leukocytosis and other non-specific effects. v. Jauregg has even suggested the advisability of inoculating with malaria in order to secure the benefit of the febrile reactions; Mühlens, Weygandt, and Kirschbaum¹⁰ have recently reported on the treatment of cases inoculated with the *Spirillum obermeieri* and malaria plasmodia, the literature being covered by Raecke.¹¹

In *tabes dorsalis* Schacherl¹² and others have employed injections of old tuberculin, beginning with 0.001 gm. and giving 0.1 gm. of salicylate of mercury with each third dose. In the treatment of 76 cases the results were interpreted as very encouraging. Others have employed injections of milk. With both agents the injections may precipitate a gastric crises or lancinating pains.

SERUM TREATMENT OF SNAKE-BITES

The nature of snake venom is discussed in Chapter VII, and the method of preparing antivenomous serum is described in Chapter XIII.

Calmette's¹³ *antivenene* for cobra venom is useful in the treatment of cobra envenomation, but is not serviceable for the treatment of other snake-bites, as shown by Martin for Australian serpents and by McFarland for American snakes. In the venoms of our snakes, as, for example, the rattlesnake, copper-head, and moccasin, the poison is essentially locally destructive, the respiratory poison being of secondary importance. McFarland¹⁴ failed to immunize horses against this locally destructive poison. Later Noguchi and Madsden¹⁵ succeeded in producing an antiserum, prepared by immunizing horses with venom after the toxophorous groups of the molecules had been destroyed, capable of neutralizing the *hemorrhagin* of the *Crotalus* venom.

¹ Wien. med. Wchn., 1909, 39, 2124; *ibid.*, 1913, 53, 2555.

² Wien. med. Wchn., 1912, 52, 2010 and 2083.

³ Neurol. Centralbl., 1913, 32, 313.

⁴ Riv. ital. d. neuropatol., etc., 1912, 5, 117.

⁵ Russk. Urach., 1913, No. 24, 862.

⁶ Wien. klin. Wchn., 1909, 22, 1289; Allg. Ztschr. f. Psych., 1910, 57, 420.

⁷ Prag. med. Wchn., 1909, 34, 401.

⁸ Neurol. Centralbl., 1916, 35, 57.

⁹ Jour. Mental Sci., 1912, 48, 389.

¹⁰ Münch. med. Wchn., 1920, 57, 831.

¹¹ Therap. Monatsh., 1920, 34, 129.

¹² Jahrb. f. Psych. u. Neurol., 1914-15, 35, 207.

¹³ Jour. Med. Research, 1909, 21, 47.

¹⁴ Jour. Med. Research, 1909, 21, 51.

¹⁵ Jour. Exper. Med., 1907, 9, 18.

The serums of Calmette, Noguchi,¹ Flexner and Noguchi,² and others are useful in the treatment of their respective envenomations, but aside from India, Brazil, and a few other reptile-infested countries, as well as in zoological gardens and laboratories where snakes are kept, the serums have a very limited sphere of usefulness.

Houssay, Sordelli, and Negrete³ have recently made extensive studies on the preparation and action of various antisera for South American snake venoms. In Brazil two specific sera (antibothropic and anticrotalic) have yielded excellent results, and a third polyvalent or antiophidian serum has recently been announced from the Serotherapeutic Institute of Brazil, which is believed to counteract the effects of all poisonous reptiles in Brazil. Similar efforts are to be made in India, where of over 300 species of snakes, at least 68 are known to be poisonous with an annual death-rate of over 20,000 human beings and countless numbers of animals.

TREATMENT OF OTHER DISEASES

Autosera Treatment of Chorea.—Sydenham's chorea or St. Vitus' Dance, possessing certain features suggestive of focal infection and especially in relation to the occurrence of arthritis in about 20 per cent. of cases, endocarditis, as well as to its occasional development after whooping-cough, acute septicemia, etc., and in dogs after distemper, has been treated by Goodman⁴ with intraspinal injections of the patient's own serum. About 40 to 50 c.c. of blood are drawn aseptically and the serum separated; spinal puncture is conducted, and after removal of 15 to 20 c.c. of fluid, an equal amount of the serum is injected very slowly. Of 30 cases treated by Goodman, 14 received one injection, 8 received two injections, and the balance three or more. In the majority of these cases twitching ceased within a week or two, but the duration of the improvement has not been stated.

Porter⁵ has also reported upon the results of this treatment, and advises doses of 5 to 10 c.c. of serum; Brown, Smith, and Phillips⁶ report the cure of 18 patients of a series of 23, with improvement in 4 and failure in 1. Seventeen of these were mild cases and 5 were severe. The average number of injections were three. Reactions usually followed the injections. Four cases were relieved in one week and 19 in three weeks. Tarr⁷ has also observed good results with this therapy.

Non-specific Protein Treatment of Epilepsy.—Döllken⁸ has treated a series of cases with injections of milk and a stock vaccine (for bacterial proteins) together with the administration of luminal. After four to six months of treatment 12 cases were free of attacks for eighteen months and 60 for one year. In 13 cases an improvement was noted.

If this therapy is tried, a child under twelve years of age may receive by intramuscular injection 1 to 2 c.c. of market milk boiled for ten minutes and cooled. Injections are given only after attacks occur, so that the intervals are irregular.

Edgeworth⁹ has reported favorably upon the intravenous injection of a sterilized 5 per cent. solution of Witte's peptone. The first dose may be

¹ Jour. Exper. Med., 1906, 8, 614.

² Jour. Med. Research, 1904, 11, 363.

³ Rivista del Inst. Bacteriol., 1917, 1, 15, 485, 565, 617; *ibid.*, 1919, 2, 151, 189, 211.

⁴ Arch. Pediat., 1916, 33, 649.

⁵ Amer. Jour. Dis. Child., 1918, 16, 109.

⁶ Brit. Jour. Dis. Child., 1919, 16, 8.

⁷ Northwest Med., October, 1917.

⁸ Berl. klin. Wchn., 1920, 47, 893, 926.

⁹ Brit. Med. Jour., 1920, 2, 780.

5 minims, and subsequent doses gradually increased unless the reaction is too severe, in which case subsequent doses should be reduced.

Treatment of Typhus Fever.—Nicolle and Blaizot¹ have immunized horses with injections of extracts of suprarenal glands and spleens of guinea-pigs dying of typhus fever. They report that the treatment of 36 cases by subcutaneous injection of this serum in dose of 10 to 20 c.c. per day resulted in more prompt defervescence, relief of delirium, stupor, and prostration, and that but 1 case died.

Györi² has used autoserum treatment with benefit in some cases; Munk³ has employed injections of normal horse-serum, peptone, and nucleohexyl.

During the Great War a number of these non-specific agents have been employed. Holler⁴ used daily intravenous injections of deuterio-albumose with good results when treatment was begun within two days of the onset. In a series of 50 cases the mortality was 6 per cent., whereas of 15 untreated cases the mortality was about 50 per cent.

Tagle⁵ has used intravenous injections of 4 to 10 c.c. of 10 per cent. solutions of peptone with a mortality of 5 per cent. in 59 cases; children received 4 or 5 c.c. Second injections were given about forty-eight hours later, and in some cases a third injection. Opazo⁶ has also employed injections of peptone.

Schultz and his colleagues⁷ and Kalberlah⁸ have employed intravenous injections of typhoid vaccine; especially good results have been reported by Daniélopolu⁹ with daily intravenous injections of hypotonic salt solution (0.065 per cent.).

Non-specific Proteins in the Treatment of Mumps and Orchitis.—In the treatment of mumps of men Salvaneschi,¹⁰ Bormamour and Bardin,¹¹ and Mallié¹² have employed subcutaneous injections of 20 c.c. or more of diphtheria antitoxin or normal horse-serum. The authors believe that these injections shortened the course of the disease, were especially valuable for the prevention of orchitis, and frequently yielded relief from pain when this complication developed.

Milk injections have not been employed, but since the intramuscular (gluteal) injection of 5 to 10 c.c. of milk is known to afford relief in epididymitis, it would appear that this simple remedy is worthy of trial in orchitis.

Serum Treatment of Yellow Fever.—Noguchi¹³ has immunized horses with pure cultures of *Leptospira icteroides* and found the immune sera very active in checking the progress of the infection in guinea-pigs. By means of this serum protection test Noguchi and Kigler¹⁴ have been able to identify the *Leptospira*.

Noguchi¹⁵ has employed the serum in the treatment of 170 human cases of yellow fever, and of 95 treated before the third day the mortality was

¹ Bull. de l'Acad. de méd., 1916, 76, 95.

² Deut. med. Wchn., 1918, 44, 677.

³ Münch. med. Wchn., 1916, lxxiii, 1239.

⁴ Med. Klin., 1917, 13, 1038.

⁵ Rev. Med. de Chile, 1919, xlvii, 413.

⁶ Rev. Med. de Chile, 1919, xlvii, 433.

⁷ Berl. klin. Wchn., 1919, 45, 1226.

⁸ Therap. Monatsh., 1918, 32, 328.

⁹ Le typhus exanthématique, Bucharest, 1919.

¹⁰ Riforma med., 1917, 33, No. 37.

¹¹ Presse méd., 1920, 28, 929.

¹² Jour. de méd. de Bordeaux, 1922, 94, 16.

¹³ Jour. Exper. Med., 1920, 31, 159.

¹⁴ Jour. Exper. Med., 1920, 32, 601.

¹⁵ Jour. Amer. Med. Assoc., 1921, 77, 181.

13.6 per cent. as compared with a mortality of 56.4 per cent. in 783 cases not treated with serum. After the fourth day of the disease the serum had no appreciable effect.

Treatment of Weil's Disease (Infectious Jaundice).—The cause of this disease is now definitely established as being the *Spirochæta icterohemorrhagica* discovered by Inada and Ido¹ in 1915.

Inada, Ido, Kaneko, and Ito² have found human convalescent serum possessing some degree of curative activity, but have succeeded in preparing immune sera by inoculation of horses with cultures of the spirochete. These sera have proved effectual in the treatment of experimentally infected guinea-pigs, and likewise in the treatment of human infections when given early in the disease. From 50 to 80 c.c. of serum are injected daily in divided doses of about 20 to 30 c.c. each; injections are made subcutaneously or intravenously. With rare exceptions the serum destroys all spirochetes in the blood and reduces their numbers in the internal organs. Of 35 serum-treated cases, the mortality was 20 per cent. as compared with the usual mortality in Japan of 30 to 48 per cent. In a subsequent report by Inada³ the mortality of 86 serum-treated cases varied from 34.7 to 38.5 per cent. as compared with a mortality of 57.1 per cent. among 72 cases treated without serum.

Döllken⁴ has employed intramuscular injections of 5 to 10 c.c. of boiled milk, and reports that the results have been good in many cases. Owing to the styptic effects of milk and its effect upon the liver metabolism, this form of treatment is deserving of consideration.

Serum Treatment of Hyperthyroidism.—Beebe⁵ has described a method of immunizing sheep with intraperitoneal injections of extracts of human thyroid glands. The serum is regarded as antagonistic to the excess toxic secretions of the gland regarded by some as present in Graves' disease. It is not thyrotoxic or thyrolytic; certainly not in the amounts administered by subcutaneous injection in the treatment of the disease.

Beebe injects about 0.5 c.c. for the first dose; subsequent injections are not given until the local reaction subsides, but are usually in amounts of 1 c.c. In acute asthenic and sthenic types the serum is injected daily for several days if the reactions permit. Beebe states that the serum has proved beneficial in at least 50 per cent. of cases.

Vaccine in the Treatment of Neuritis (Sciatica).—Some cases of sciatica are now known to be due to focal infection, and especially abscesses in the roots of teeth. In sciatica and other types of neuralgia and neuritis careful search should be made for foci of infection: teeth, tonsils, accessory nasal sinuses, etc. If a focus is found and is amenable to culture and treatment, the preparation and administration of an autogenous vaccine may prove beneficial, as reported by Zapffe⁶ in sciatica.

Certain non-specific proteins have been used with success. Döllken⁷ has treated large numbers of cases of neuralgias (trigeminal, intercostal, sciatic) and neuritides of varying etiology (cold, alcoholic, post-typhoidal, syphilitic, diphtheric, etc.) with an autolysate of staphylococci and *B. prodigiosus* designated as "vaccinurin." Pressure neuralgias and so-called rheumatic palsies were most easily influenced. Holtz⁸ also observed good

¹ Tokyo Ijishinshi, 1915, No. 1908.

² Jour. Exper. Med., 1916, 23, 377; *ibid.*, 1916, 24, 485; *ibid.*, 1918, 27, 283.

³ Japan Med. World, 1922, 2, 189.

⁴ Berl. klin. Wchn., 1919, 46, 226.

⁵ Jour. Amer. Med. Assoc., 1915, 64, 413.

⁶ Jour. Amer. Med. Assoc., 1915, 64, 238.

⁷ Berl. klin. Wchn., 1914, li, 1807, 1841.

⁸ Deutsch. med. Wchn., 1918, xlv, 291.

results with this treatment. Cadbury¹ and Boyd² have used typhoid vaccine, the latter by intravenous injection.

I have treated 3 cases of sciatica and 2 of neuritis with intragluteal injections of 5 c.c. of market milk boiled for ten minutes and cooled. Marked local reactions occurred during the following twenty-four hours, but in all instances there was a prompt, but temporary, relief from pain.

If typhoid vaccine is employed, about 50,000,000 may be injected intravenously. The usual reaction of chills and fever follows, and in some cases temporary exacerbation of the neuritis.

Treatment of Marasmus.—Several reports in literature indicate that the subcutaneous injection of various protein agents, as human ascites fluid, horse-serum (normal or diphtheria antitoxin), milk, and stock vaccines may prove of distinct aid in the treatment of marantic children. The effects are purely non-specific, probably improve metabolism and digestion, and the leukocytosis may aid in overcoming latent bacterial infections, especially of the intestinal tract.

Leavy and Hastings³ and Carter⁴ for example, have given daily subcutaneous injections of 10 to 20 c.c. of sterile ascites fluid from cases of cardiac or renal disease. Slawik⁵ has employed intramuscular injections of 1 to 5 c.c. of boiled milk. Ferreira,⁶ Czerny and Eliasberg⁷ have used subcutaneous injections of horse-serum; Plantenga⁸ has employed injections of a serum prepared by immunizing animals against colon bacilli, and Putzig⁹ subcutaneous injections of diphtheria antitoxin. All of these, however, have the objection of producing allergic sensitization to horse-serum proteins with the possibility of producing serum sickness.

If the administration of non-specific agents is employed for their possible beneficial effects I believe that preference should be given intramuscular injections of boiled milk in dose of 1 or 2 c.c., sterile ascites fluid or sterile normal human serum in the same amounts; the injections may be given at intervals of one week. Very probably a stock vaccine of colon bacilli may yield similar results when injected in dose of 0.1 c.c. of a vaccine containing about 500,000,000 per cubic centimeter.

TREATMENT OF DISEASES OF THE LOWER ANIMALS

Vaccines are not widely employed for the treatment of diseases of the lower animals, although as presented in Chapters XXXV and XXXVIII, vaccines and sera are employed in the prophylaxis of many diseases. Immune sera are employed in the treatment of some diseases, notably hog cholera, anthrax, navel ill, etc. Doubtless a part of the beneficial results are due to non-specific effects, but veterinarians have not employed non-specific agents, as injections of milk, peptone, vaccines, etc., as is practised in human medicine.

Serum Treatment of Hog Cholera.—Hog cholera serum is usually employed for prophylactic immunization, as discussed in Chapter XXXVIII.

For *curative purposes* the serum has yielded good results, providing it is administered not later than the fourth day after the animal shows evidences of the disease. Several injections may be required, and the intramuscular route should be chosen because quicker absorption is thus insured.

¹ China Med. Jour., 1919, 33, 213.

² Jour. Lab. and Clin. Med., 1919, 5, 88.

³ Boston Med. and Surg. Jour., 1910, clxiii, 293.

⁴ Amer. Jour. Med. Sci., 1911, 142, 241.

⁵ Jahr. f. Kinderh., 1919, xc, 231; *ibid.*, 1921, 94, 192.

⁶ Arch. Latin-Amer. d. Pediat., 1920, 14, 109.

⁸ Geneesk. Bladen, 1918, 19, 347.

⁷ Monatschr. f. Kinderh., 1920, 18, No. 1.

⁹ Berl. klin. Wchn., 1921, 48, 151.

The use of the serum for curative purposes is, however, quite limited, because in most instances cholera hogs are destroyed in order to prevent the spread of the infection. Even though a young hog recovers, it usually remains a "runt," growing poorly and remaining underweight. The serum, however, may be used for the treatment of particularly valuable animals and those diagnosed very early in the disease and showing nothing but a rise of temperature.

Serum Treatment of Rinderpest.—As mentioned in Chapter XXXVIII, this disease (cattle plague) is prevalent among the cattle of European and African countries; the etiologic agent is present in the blood and bile, and is filtrable. Kolle and Turner have prepared immune serum by injecting healthy oxen subcutaneously with increasing amounts of infectious blood and bile. The serum is said to be curative when administered by subcutaneous or intramuscular injection at least thirty days after the onset of fever. Of 3318 animals treated in this manner, 455, or 13.9 per cent., died, while the mortality among non-treated animals is very high, averaging 85 to 95 per cent.

Serum Treatment of Anthrax.—The Department of Agriculture¹ has given the following directions for the administration of anti-anthrax serum for curative purposes:

"In the treatment of anthrax serum should be administered in large doses. An animal showing only a high temperature, with no other manifestations of the disease, should be given from 30 to 50 c.c., but if the gravity of the disease is pronounced, 100 c.c. should be administered. In almost every instance a drop in temperature may be observed and a diminishing of the severity of the symptoms. At times, however, a relapse occurs about the second or third day following the serum injection, when it becomes necessary to administer another and larger dose of serum. It has been proved that animals affected with anthrax, even after the bacilli are found in the blood circulation, may recover after a large injection of potent serum. There is no danger from large doses—the danger is in insufficient dosage or delay in administration."

In administering the serum for curative purposes injections should be made intravenously for quick results, and repeated as often as apparently needed. If intravenous injection is impossible, the serum should preferably be injected at two or three different places subcutaneously to facilitate rapid absorption.

Serum Treatment of White Scours.—For the treatment of larger animals 30 to 100 c.c. of immune serum may be given intravenously upon the first warning of the disease by the presence of blood in the feces; hogs, sheep, and goats may receive 10 to 20 c.c. and cats and dogs about 5 to 10 c.c. When intravenous injections cannot be made, the serum should be injected intramuscularly. A second intramuscular injection may be given twelve hours later and thereafter at intervals of twenty-four to forty-eight hours until the disease is checked.

Serum Treatment of Joint-ill of Colts.—An immune serum prepared by the immunization of horses with *Bacillus abortus-equi* and streptococci has been used successfully in the treatment of arthritis, joint-ill, or navel-ill of colts; the usual dose is 5 to 20 c.c. by subcutaneous injection repeated as necessary. M'Fadyean and Edwards² have observed encouraging results with this form of treatment.

Serum Prophylaxis and Treatment of Hemorrhagic Septicemia.—The

¹ U. S. Dept. of Agricult. Bull. No. 340, December 27, 1915.

² Jour. Comparative Path. and Therap., 1917, 30, 321.

bacteriology and vaccine prophylaxis of hemorrhagic septicemia or pasteurellosis of animals (horses, cattle, hogs, sheep, etc.) have been discussed in Chapter XXXV. Antisera are prepared by the immunization of horses with the different varieties of *Bacillus bipolaris septicus* and their toxins.

For *prophylactic purposes* 20 to 40 c.c. may be injected subcutaneously; the immunity, however, disappears after six weeks.

For *curative purposes* 30 to 100 c.c. are required, according to the size of the animal and severity of the disease. If possible, the serum should be injected intravenously; otherwise, intramuscularly. Subsequent injections may be made intramuscularly as required.

Serum Prophylaxis and Treatment of Equine Influenza and Strangles.—

The bacteriology and vaccine prophylaxis of these diseases have been discussed in Chapter XXXV. Antisera are prepared by the immunization of horses with pneumostreptococci and *Bacillus equisepticus* of equine influenza and with *Streptococcus equi* (Schütz) of strangles.

For *prophylactic immunization* 30 to 50 c.c. may be injected subcutaneously or intravenously; the passive immunity gradually disappears after three weeks.

For *curative purposes* 50 to 100 c.c. should be injected intravenously or intramuscularly; subsequent doses may be given by intramuscular injection as required.

CHAPTER XLI

BIOLOGIC THERAPY OF TUBERCULOSIS

IMMUNITY IN TUBERCULOSIS

ALL human beings are susceptible to tuberculosis and the disease is always found where opportunity for infection is present. The very widespread dissemination of the disease throughout the world and the frequency with which tubercle is found in necropsies indicates that natural immunity to tuberculosis probably does not exist. Not all human beings, however, become infected with tubercle bacilli; probably 5 to 10 per cent. escape, in so far as finding evidence of the disease after death is concerned, and especially in necropsies of individuals living in the rural districts. But approximately 90 per cent. of adult human beings are said to show evidences of tuberculous infection, although, fortunately, in the majority of these the disease remains strictly localized, undiscovered during life, and produces no symptoms.

Tuberculosis also occurs among the lower animals, and especially cattle, but these animals apparently possess a greater degree of natural immunity than man. Hogs are sometimes infected (2.5 per cent.), but horses, sheep, and goats possess a very high natural immunity and are seldom infected. Tuberculosis also occurs among the cold-blooded animals, but these bacilli do not cause more than local lesions when injected into warm-blooded animals, and, indeed, may fail to produce any infection. Likewise tubercle bacilli of warm-blooded animals usually fail to infect cold-blooded animals.

For human beings human tubercle bacilli are most infective; there is a greater degree of natural immunity to bovine bacilli, but it is now generally agreed that the latter may infect man and especially the lymph-glands of children. Likewise the lower animals possess a high natural immunity to human tubercle bacilli and are rarely infected spontaneously with these organisms.

Certain families and individuals appear to possess a certain degree of natural immunity, but it is always difficult and usually impossible during life to absolutely exclude the presence of latent foci capable of engendering some degree of acquired immunity. For example, Petruschly¹ calls attention to "mother immunity," meaning that a woman from a non-tuberculous family apparently escapes infection from her tuberculous husband and yet her children succumb to tuberculosis. These mothers usually react to tuberculin, indicating the presence of latent infection, and the condition is in some respects analogous to women giving birth to syphilitic children without themselves showing active lesions of syphilis, but nevertheless luetic, and showing the acquired resistance to reinfection with *Spirochæta pallida* as a result of latent syphilis.

Children of tuberculous parents do not contract tuberculosis *in utero*; the disease is not a prenatal infection, as may occur in syphilis. But these children likewise do not inherit an immunity to infection. On the other hand, the death-rate among these children may be relatively low because they are especially apt to contract the disease early in life and acquire the resistance conferred by tuberculous foci. If, however, these children are

¹ *Ergebn. d. Immunitätsf.*, 1914, 1, 189.

repeatedly exposed to massive infections in a household, this acquired immunity is readily broken down with a consequently high death-rate.

Of course, not all individuals exposed to tuberculosis acquire the infection. Doubtless in contamination of the mucous membranes of the upper respiratory tract with tubercle bacilli of attenuated virulence the organisms are removed mechanically by the secretions, and their entrance into the tissues and lymphatic channels blocked by epithelial barriers. Furthermore, it is highly probable that fixed and wandering cells may destroy the bacilli by phagocytosis even though the blood and lymph possess very feeble tuberculocidal properties.

Why human beings possess a high natural immunity for bovine tubercle bacilli is not known; likewise the nature of the resistance of the warm-blooded animals to tubercle bacilli of the cold-blooded animals and the reverse are unsolved. Nothing in the way of immunity principles are found in the blood and lymph to account for this natural resistance. The bacilli when injected in massive doses may produce local inflammatory lesions, but these are similar to lesions produced by any bacterial protein. The bacilli simply fail to survive and multiply as if lacking a suitable pabulum and suggesting the operation of a mechanism of athreptic immunity, offered by Ehrlich as an explanation for the failure of some mouse cancers to grow in rats, and the reverse. Probably repeated and persistent cross-infection tests would result in the bacilli acquiring the property of surviving in the tissues of a heterologous host and resisting the destructive action of phagocytosis. Furthermore, human tubercle bacilli show a marked predilection for certain issues, notably lymphoid tissue, as if finding there favorable conditions for survival and multiplication. They seldom infect the muscles and may leave no traces of reaction in the tissues of entry, as the lungs or intestinal mucosa. This selective affinity may have something to do with the question of pabulum or, more probably, with chemical conditions influencing their growth and metabolism; as long ago as 1889 Weber¹ suggested that deficiency of carbon dioxid in the tissues favors tuberculosis, while accumulation retards the bacilli, and Corper, Gauss, and Rensch² have recently reported experimental evidence in support of this empiric contention.

Even though our natural defenses against tuberculous infection may be insufficient for affording complete protection, yet they are sufficient for rendering the initial infection a strictly localized affair and usually of the lymph-glands. It is very doubtful that acute overwhelming miliary tuberculosis occurs spontaneously as the primary infection.

When a few bacilli of moderate virulence gain access to susceptible tissues the mechanism of defense is purely cellular. As shown so clearly by Foote³ the vascular endothelial cells proliferate and migrate, producing the cells designated as "epitheliod," which surround, wall off, and phagocytose the bacilli. There may be no other inflammatory vascular phenomena and infiltration with leukocytes, and the complete destruction of the bacilli is apparently accomplished by these means.

With larger numbers of bacilli or organisms of greater virulence the local reaction is much the same plus inflammatory changes. There may be an early and temporary infiltration with polymorphonuclear leukocytes followed by proliferation and migration of vascular endothelial cells. The latter may multiply and fuse in the lesion to form the giant-cells. The

¹ Therap. Monatsh., 1901, 15, 130.

² Jour. Amer. Med. Assoc., 1921, 76, 1216.

³ Jour. Exper. Med., 1920, 32, 513, 533; *ibid.*, 1921, 33, 271; Jour. Med. Research, 1919, 40, 353.

cells of the part coming in closest contact with the bacilli are apt to undergo destruction. The main struggle is apparently between the invading bacilli and the defensive endothelial cells, the latter aiming to wall off the bacilli and attempting their destruction to some extent by phagocytosis. Later the lymphocytes migrate from the surrounding vessels and the connective-tissue cells proliferate into fibroblasts with the production of a wall composed of all of these elements. This wall is the chief means of defense against the spread of the infections. As stated by Krause,¹ "the patient is as resistant as the shell of his tubercle." Within this shell the bacilli may survive for long periods of time; their toxins gradually destroy the cells with which they come in contact, producing caseation necrosis, which may finally undergo enzymic digestion or liquefaction necrosis. The products of the bacilli plus those of the necrotic cells constitute the foci poisons; the absorption of these is responsible for the general symptoms of tuberculosis, as pyrexia, tachycardia, anemia, etc.

These changes may be progressive and effectually resist the walling off process. Anything favoring the production of fibrous tissue favors the localization of the disease or clinical cure. In this connection mild passive hyperemia is probably beneficial as favoring fibrous tissue production, and forms the basis for the tuberculin treatment of some forms of tuberculosis in order to secure the beneficial effects of the focal hyperemia of the allergic reaction. At the same time, however, anything producing excessive hyperemia, as excessive amounts of tuberculin, influences wear and tear upon the encapsulating tissue and may favor multiplication of bacilli, and more especially facilitate their spread by continuity of tissues, by migrating endothelial cells, or by vascular and lymphatic channels.

To a limited extent the newly formed endothelial cells of a tubercle are capable of destroying bacilli of low virulence by means of phagocytosis and endocellular ferments; likewise the products of tissue necrosis and especially liberated enzymic substances may have a bacteriostatic and bactericidal effect and bring about the sterilization of the tubercle in a manner analogous to the autosterilization of abscesses produced by gonococci and other pyogenic bacteria. Certainly resistance is cellular and largely mechanical in primary infections with tubercle bacilli. Since lymphocytes are found so abundantly in tubercles and are increased in the blood it would appear that they must be also one of nature's defensive agencies. Murphy and Ellis² have observed that mice subjected to exposure to x-rays are rendered more susceptible to tuberculosis and these effects were ascribed to the destruction of lymphocytes and lymphoid tissue. Morton³ has employed x-rays for hastening the development of tuberculosis in guinea-pigs, but Weinberg⁴ was unable to hasten the progress of tuberculosis appreciably by exposure of the guinea-pig to massive doses of the x-rays, although there occurred a marked reduction in the numbers of lymphocytes in the blood. White and Gammon⁵ have also reported that the inhalation of benzene by rabbits reduces their resistance to tuberculosis. The investigations of Hektoen, employing x-rays and other substances for leukocytic and lymphoid tissue destruction, have indicated that these tissues are concerned in antibody production to different antigens (Chapter VIII).

The rôle of small lymphocytes in resistance to tuberculosis is doubtful, but evidence indicates that they probably play a purely secondary part. They apparently are not actively phagocytic; they arrive at the scene of

¹ Johns Hopkins Hosp. Bull., 1917, 28, 191.

² Jour. Exper. Med., 1914, 20, 397.

³ Jour. Exper. Med., 1916, 24, 419.

⁴ Arch. Int. Med., 1920, 25, 565.

⁵ Tr. Assn. Am. Phys., 1914, 19, 332.

battle rather late and take up a position somewhat to the rear. Doubtless they are of most assistance as wall builders and may contribute to the production of the fibrous sheath. At all events, the fact that tubercle bacilli find lymphoid tissue their choice of location and operation indicates that there can be little actual antagonism between the tubercle bacillus and small lymphoid cells. The bacillus exercises an attraction for them—a positive chemotaxis similar to the positive chemotaxis for these cells displayed in syphilis and other chronic infections.

It would appear, therefore, that in primary tuberculosis, when bacilli of sufficient virulence have succeeded for the first time in invading our tissues and producing tubercle, the resistance offered them is almost purely cellular. Glandular secretions may sweep them away mechanically, but are not bactericidal. Doubtless the blood is feebly restraining and destructive as it is for most organisms, but these activities are of little importance. The lymph apparently is even less antagonistic. But after the tubercle has been formed an increased resistance to reinfection becomes manifest in which the cells again play the major rôle in resistance, but in which the body fluids also participate in a more important manner.

Now the invasion of tubercle bacilli is followed by an accelerated inflammatory reaction characterized by hyperemia, cellular, and more particularly by serous exudation. These changes are a result of the primary infection and may occur in tissues remotely removed from the primary lesion; they occur only when a focus of living tubercle bacilli has been established, and decrease with healing, but seldom disappear entirely because the complete destruction of all living bacilli is of infrequent occurrence.

This reaction to reinfection or the administration of dead bacilli or their products (tuberculins) is the allergic reaction of tuberculosis. Sensitization of the tissues of the tubercle and of the tissues in general cannot be brought about by anything short of living tubercle bacilli, and hence the failure of prophylactic immunization against tuberculosis by vaccines of dead bacilli and tuberculins. Indeed, as shown by Romer and Joseph,¹ acquired resistance requires the presence of a focus of infection and may not be gained by careful immunization even with vaccines of living bacilli, not to mention vaccines of dead bacilli. The mechanism of the reaction has been previously discussed in Chapter XXIX and is probably a cellular colloidal phenomenon.

Krause has regarded this reaction as an important means of defense against reinfection. In all probability the allergic antibodies in the cells are not actually destructive for tubercle bacilli, but the inflammatory reaction is, and probably by reason of facilitating phagocytosis and to some extent by bringing into play plasma of enhanced tuberculocidal activities, as at this time small amounts of various antibodies may be found in the blood. Krause and Hofer² have found that bacilli injected into the peritoneal cavity of tuberculous guinea-pigs were quickly destroyed by lysis; Manwaring and Bronfenbrenner³ have also studied this phenomenon and found that whereas tubercle bacilli injected into the peritoneal cavities of normal guinea-pigs may be recovered, those injected into tuberculous animals sometimes underwent lysis ascribed to acquired changes in the fixed peritoneal cells. In so far as resistance and immunity are concerned the most important of these antibodies are opsonins and lysins, while agglutinins and precipitins may aid in both processes of phagocytosis, intracellular and extracellular lysis.

¹ Beitr. z. Klin. d. Tuberk., 1910, 17, 281.

² Deutsch. med. Wchn., 1912.

³ Trans. Nat. Assoc. Study and Prevent. Tuberculosis, 1913, 321.

Antibodies in the blood, however, are probably never the important factors of acquired resistance to tuberculosis. Complement-fixation and other tests have shown that these seldom reach a high point of production. The chief factors are apparently the cells and phagocytosis.

Probably the majority of human beings after fifteen years owe their freedom from clinical tuberculosis to the presence of small latent foci bringing about this sensitization and accelerated and abortive reactions upon reinfection. In 1886 Marfan¹ announced the following "law" in tuberculosis: "One almost never finds pulmonary tuberculosis, at least manifest and a progressive disease, in people who in infancy have been the subjects of scrofula (suppurative tuberculous adenitis of the neck) and who have been completely cured of this before the age of fifteen, such cure having taken place before any other focus of tuberculosis was discoverable."

Children may be overwhelmed before the mechanism is well under way, and in both children and adults this resistance is readily enough broken down by massive reinfection and auto-infection, as from a broken-down focus followed by the discharge of large numbers of virulent bacilli which are now probably endowed with more effectual means of resisting the defensive agencies of the host. Calmette² has observed that cattle nearly always recover from a single infection if carefully isolated, whereas they rarely recover, but become actively tuberculous, if they are infected several times at short intervals, or if they are left in prolonged contact with tuberculous animals. Calmette concludes that the consumptive is one who has received these repeated and successive reinfections capable of overcoming the resistance acquired with the primary infection.

In syphilis we have seen that when the disease is established there is apparently an almost absolute resistance to reinfection until cure is accomplished, when susceptibility is restored, but, unfortunately, this is not so in tuberculosis. Instead of nearly absolute acquired resistance the resistance is only relative. While the syphilitic is highly resistant to reinfection, he is not immune to progressive involvement of his own tissues by *Spirochæta pallida*; the tuberculous individual is much less resistant to reinfection, but his primary lesion may not progress and, indeed, in most individuals does not do so to any great extent. In this respect tuberculosis is much less dangerous. In both diseases the parasites may survive indefinitely, but in tuberculosis are more effectually restrained than occurs in syphilis.

It is very difficult to determine whether this acquired resistance to tuberculous reinfection persists after destruction of all tubercle bacilli in the body, and if so, how long it persists. As previously stated, the resistance is not transmitted *in utero* and can be passively transferred by injection of serum only to a slight, uncertain, and temporary degree. It is a resistance dependent upon the sensitizing activity of some allergen associated in some way with living bacilli in the tissues, but it is highly probable that the protective tissue sensitization persists in a gradually decreasing degree for some time after complete destruction or removal of the living bacilli, and to this extent it may be stated that an attack of tuberculosis confers a certain measure of temporary immunity after recovery. Since living bacilli have been found in calcified foci it is highly probable that complete destruction of all tubercle bacilli even in a small isolated lymph-gland is the exception rather than the rule, and may never occur at all; we are usually unable to destroy the invading enemy, but we may protect ourselves against his multiplication and further invasion and against superinfection.

¹ Arch. gén. de méd., 1886, 1.

² L'Infection Bacillaire et la Tuberculose, Masson and Cie, Paris.

PROPHYLACTIC IMMUNIZATION IN TUBERCULOSIS

With a disease so wide-spread as tuberculosis it is natural that early and persistent attempts should be made for developing a practical and efficient means of prophylactic immunization. Without doubt this accomplishment would be among the greatest of triumphs in medicine.

Koch originally stated that only living tubercle bacilli producing a mild infection could accomplish immunization, and the sum and substance of a great deal of investigation since then, recently summarized by Neufeld,¹ has only served to emphasize the truth of this assertion.

As stated above in the discussion of the nature of resistance and immunity in tuberculosis, the presence of a lesion is required to bring about the sensitization of the tissues necessary for the enhanced abortive and inflammatory reactions regarded as allergic and capable of offering resistance to reinfection with bacilli of reduced virulence or within certain small dosage. Even this degree of resistance, which is the best obtainable in tuberculosis, is not always able to hold the individual's own infection in check, and may be broken down or overcome by infection with "massive" numbers of tubercle bacilli or by small numbers of very virulent bacilli.

Injections of dead bacilli and extracts do not produce this protective sensitization of the tissues; they may bring about anaphylactic sensitization to the proteins of the tubercle bacillus, but this process is not protective, and anaphylactized animals are susceptible and, indeed, may be somewhat more susceptible to infection than normal control animals. This subject has been discussed in more detail in the chapters devoted to Anaphylaxis and Allergy.

If an actual focus of disease so small as to be symptomless is capable of engendering some degree of resistance to tuberculosis, what is to be expected of immunization with non-virulent living tubercle bacilli or of virulent bacilli in very small numbers?

Probably the first work done with the living bacillus was that of Dixon² with attenuated cultures. This worker found that animals inoculated with an old culture containing club-shaped and branching forms of tubercle bacilli would resist subsequent inoculation with virulent organisms. Since then numerous investigators—Trudeau,³ Pearson,⁴ de Schweinitz,⁵ McFadyen,⁶ Levy,⁷ Pearson and Gilliland,⁸ Behring,⁹ Thomassen,¹⁰ Neufeld,¹¹ Theobald and Smith,¹² Webb and Williams,¹³ and others—have, either directly or indirectly, supported this original work, thus indicating that the most effectual active immunization is secured by using living cultures. The method employed by Webb and Williams is worthy of special mention, inasmuch as the ascending doses of bacilli are actually counted by an ingenious method devised by Barbour.¹⁴ These observers used this method quite ex-

¹ Ztschr. f. Tuberkulose, 1921, 35, 11.

² Medical News, Philadelphia, October 19, 1889.

³ Amer. Jour. Med. Sci., August, 1906; June, 1907; New York Med. Jour., July 23, 1893; Medical News, October 24, 1903.

⁴ Proc. First Internat. Vet. Congress, 1893.

⁵ Medical News, December 8, 1894.

⁶ Jour. of Comparative Path. and Therap., June, 1901; March, 1902.

⁷ Centralbl. f. Bakt., 1903.

⁸ Phila. Med. Jour., November 29, 1902; Univ. of Penna. Med. Bull., 1905.

⁹ Beitr. z. exper. Therap., Reft. s. Marburg, 1902.

¹⁰ Recui' de Med. Vet., January 15, 1903.

¹¹ Deutsch. med. Wchn., September 1, 1902; April 28, 1904.

¹² Jour. Med. Research, June, 1908.

¹³ Trans. of the Sixth Internat. Congress on Tuberculosis, 1908; Jour. Med. Research, 1911, xix 1.

¹⁴ The Kansas Univ. Sci. Bull., 1907, iv, No. 1.

tensively with lower animals, and have also secured good results with persons willing and anxious to take all possible risks for the possible good that may result. In no case have harmful results followed the injections.

Naturally, most experiments on prophylactic immunization against tuberculosis have been conducted with the lower animals, and notably cows, by reason of the special value such experiments possess from the standpoint of milk production and the prevention of bovine infection of human beings.

Von Behring has employed on an extensive scale the immunization of cattle with intravenous injections of living human bacilli because these organisms are usually non-virulent for adult cattle, although calves are sometimes infected. The outcome of these experiments showed that some degree of resistance was attained, but that it was temporary and apparently dependent upon the duration of the living bacilli of the vaccine in the tissues. Theobald Smith¹ in repeating experiments of this kind also noted the development of a marked degree of resistance to subsequent infections with bovine bacilli, but likewise found that the resistance was temporary and that the method was not without danger, inasmuch as the living bacilli of the vaccine may persist for unknown periods of time in the tissues and in the presence of animal parasites, injuries and inflammatory lesions due to other infections multiply and produce tuberculous lesions.

A very complete review of the literature on prophylactic immunization of cattle is given by Gilliland²; also an account of the extensive experiments conducted by him and his associates in Pennsylvania upon the immunizing value of intravenous injections of tubercle bacilli from human sources non-virulent for cattle. The conclusions drawn from this extensive and very carefully conducted investigation are as follows:

I. Intravenous injections of tubercle bacilli from human sources non-virulent for cattle are capable of conferring an immunity in cattle against tuberculosis sufficient to withstand natural infection by association with tuberculous cows.

II. The length of the immunity conferred has not been determined definitely, but it is believed to gradually diminish after two and one-half years.

III. The vaccinated animal during the period of vaccination and for some weeks afterward is more liable to contract tuberculosis than a normal animal. The natural resistance of the animal is apparently lowered during the time of vaccination.

IV. The interval between the vaccinations should be of a sufficient length to allow any reaction following the previous vaccination to entirely subside.

V. The degree of immunity obtained in the animal depends to a certain extent upon the number of vaccinations and the amount of vaccine administered.

VI. The vaccine should be prepared so it contains no clumps of bacilli and should be administered fresh.

VII. A number of the vaccinated animals may give a typical tuberculin reaction following the vaccinations for a period of twenty months. These animals may or may not show lesions of tuberculosis at autopsy.

VIII. Vaccine administered to animals already infected with tuberculosis is capable of retarding or holding in check the progress of the disease.

IX. The milk from immunized cows when fed over a long period of time appears to increase the resistance of calves and pigs.

¹ Jour. Amer. Med. Assoc., 1917, 68, 764.

² Circular No. 32 of the State Livestock Sanitary Board of Pennsylvania, Harrisburg, 1915.

X. Vaccination of calves against tuberculosis is of assistance in the eradication of tuberculosis from a herd if done under the proper conditions.

XI. Until further knowledge is obtained in regard to the destruction or outcome of the living tubercle bacilli constituting the vaccine, no practical method for the immunization of milk-producing animals under ordinary conditions can be advocated.

Recently Calmette and Guérin¹ have reported that immunization of heifers with a vaccine of living bovine bacilli attenuated by prolonged cultivation on media containing bile confers an immunity to infection by swallowing living bacilli from cattle or by intravenous injection. Calmette believes that human tuberculosis is usually infection with bovine bacilli and that the usual route of infection is gastro-intestinal. A great deal of work the world over has shown, however, that the majority of cases of tuberculosis in human beings are due to human types of bacilli, and that infections with bovine bacilli are largely met with in children. However, their claim that immunization of cattle with this vaccine of attenuated bacilli protects animals against tuberculosis and that if it is used upon a sufficiently extensive scale, tuberculosis in cattle will disappear, and that with its disappearance tuberculosis in man will diminish, and finally disappear, cannot be said to be proved by any means. The number of animals employed by them was far too small for such sweeping statements, but the results are noteworthy and encouraging and should stimulate further work along similar lines.

Within recent years the profession and laity have also been agitated by the extravagant claims of Friedmann for a vaccine of living, acid-fast bacilli derived from the turtle. This culture is said to be avirulent for human beings, and to be capable of stimulating specific antibodies and thus bringing about a cure. The unfortunate methods by which these claims have been exploited, and the investigations by Anderson and Stimson² and others tending to show that no good has followed its use, and that the vaccine is not entirely harmless, preclude any statements at this time except to state that the vaccine has not demonstrated powers of prophylactic immunization in well-controlled experiments.

Other non-virulent living vaccines have been advocated for prophylactic immunization of human beings and cattle. It is claimed that their administration is followed by the development of antibodies, but none have withstood the acid test of preventing infection with virulent bacilli with sufficient regularity or for sufficient lengths of time to render them of practical value. The principle involved, however, namely, the use of a living vaccine composed of bacilli so altered by passage through another animal or by chemical and physical agents, that they cannot produce tuberculosis, but yet resemble the infecting bacillus closely enough to produce protection, is sound, and should stimulate further investigation in this direction. Certainly enough work has been done to show that very little or nothing of value is to be expected of vaccine of dead bacilli and various tuberculins. Just as in vaccination against smallpox the living organism is required either in the form of a purposely induced mild infection or vaccination with a modified, but living virus, the same are apparently required for immunization in tuberculosis and syphilis. The first procedure is out of the question because the infection cannot be controlled, and the second, up to the present time, has been found to confer only temporary resistance and to be not without some danger.

¹ Ann. de l'Inst. Pasteur, 1911, 25, 625; *ibid.*, 1920, 34, 553.

² Hygienic Lab. Bull. No. 99, 1914.

THE PRINCIPLES OF TUBERCULIN THERAPY

Historic.—Within the last twenty years the subject of tuberculin therapy has elicited considerable discussion in the diagnosis and treatment of tuberculosis. The wide-spread prevalence of the disease not only in man, but in the lower animals as well, the distressing symptoms, the gloomy prognosis, and the economic importance it possesses, are a few of the factors that have stimulated investigators the world over to zealous and persistent efforts directed toward discovering a means of preventing and curing this great scourge. Owing to the nature of the infection, which covers relatively long periods of time, and the fact that much time is required for the conduct of experimental studies, researches are of necessity prolonged, tedious, and difficult. Within a period of a few years after the cause of syphilis had been discovered and isolated valuable diagnostic reactions and a well-nigh specific therapy were discovered. The discovery of an early and specific diagnostic and therapeutic measure for tuberculosis will achieve still greater triumphs—in fact, few could be greater or more beneficial.

Koch was the first to note the curative action of tuberculin, and it may be well to refer here to the original description of his fundamental experiments,¹ which have been the basis as well as the starting-point of the entire study of tuberculin:

“When one vaccinates a healthy guinea-pig with a pure culture of tubercle bacilli the wound, as a rule, closes and in the first few days seems to heal. However, in from ten to fourteen days a hard nodule appears which soon breaks down, leaving an ulcer that persists to the time of death of the animal. There is quite a different sequence of events when a tuberculous guinea-pig is vaccinated. For this experiment animals are best suited that have been successfully infected four to six weeks previously. In such an animal the inoculation wound likewise promptly unites. However, no nodule forms, but on the next or second day after a peculiar change occurs. The point of inoculation and the tissues about, over an area of from 0.1 to 1 cm. in diameter, grow hard and take on a dark discoloration. Observation on subsequent days makes it more and more apparent that the altered skin is necrotic. It is finally cast off, and a shallow ulceration remains, which usually heals quickly and permanently without the neighboring lymph-glands becoming infected. Inoculated tubercle bacilli act very differently then upon the skin of healthy and tuberculous guinea-pigs. This striking action is not restricted to living tubercle bacilli, but is equally manifested by dead bacilli, whether they be killed by exposure to low temperature for a long time or to the boiling temperature, or by the action of various chemicals.

“After having discovered these remarkable facts, I followed them up in all directions and was further able to show that killed pure cultures of tubercle bacilli ground up and suspended in water can be injected in large amounts under the skin of healthy guinea-pigs without producing any effect other than local suppuration. Tuberculous guinea-pigs, on the other hand, are killed in from six to forty-eight hours, according to the dose given, by the injection of small quantities of such a suspension. A dose which just falls short of the amount necessary to kill the animal may produce extensive necrosis of the skin about the point of injection. If the suspension be diluted until it is just visibly cloudy the injected animals remain alive, and if the administration is continued with one- to two-day intervals, a rapid improvement in their condition takes place; the ulcerating inoculation wound becomes smaller and is finally replaced by a scar, a process that

¹ Deutsch. med. Wchn., 1891, xvii, 101.

never occurs without such treatment; the swollen lymph-glands become smaller; the nutrition improves, and the disease process, unless it is too far advanced and the animals die of exhaustion, comes to a standstill.

"Thus was established the basis for a rational treatment of tuberculosis. However, such suspensions of killed tubercle bacilli are unsuitable for practical use, since they are neither absorbed nor disposed of in other ways, but remain a long time unaltered at the point of inoculation and occasion smaller or larger abscess."

Koch showed further that while the injection of tuberculous guinea-pigs with large doses of tubercle bacilli produced rapid death, frequently repeated small doses exerted a favorable effect upon the site of infection and the general condition of the animals. The same observer also realized that the harmful effects of injections of dead tubercle bacilli were due to the non-absorbable parts of the bacilli. He attempted to extract the immunizing substances, and in this way produced his first or old tuberculin. When injected into tuberculous guinea-pigs, old tuberculin produced a rapid general reaction without any local necrosis or sloughing, whereas when injected into a healthy guinea-pig, no reaction, either local or general, was produced. The fact that the general results produced by old tuberculin were analogous to those obtained by his first vaccine, except that local necrosis did not occur, induced Koch, in 1891, to promulgate it as a specific cure for tuberculosis in human beings.

It is hardly necessary to describe the hopeful anticipation with which it was received, and the keen disappointment that followed its earlier clinical use. Indiscriminate use, extravagant expectations, and excessive dosage combined to yield results so discouraging as to swing the pendulum of medical opinion so far the other way that even now the very word "tuberculin" suggests to many minds failure and something to be avoided.

A few earlier followers of Koch continued their studies in the endeavor to discover the causes of failure in tuberculin therapy. Their researches have led to new principles in treatment and to more exact knowledge of its indications, as well as its contraindications. As now employed, its use being restricted to suitable patients and administered in safe graduated doses, and accepting as evidence only the statements of those who have used tuberculin and not of those who believe it to be dangerous and have never used it, one deduction is justified: that while tuberculin is not a specific "cure" for tuberculosis—any more than hygiene, diet, and climate are cures—it helps to arrest the disease and is in general a useful factor in the treatment of certain types of the disease. Clinical studies have shown, however, that immunization of the tuberculous patient is frequently a difficult procedure, owing to the fact that such patients are prone to develop a remarkable state of hypersusceptibility, in consequence of which every inoculation will produce a reaction that may be injurious to the patient.

Nomenclature.—All extracts and suspensions of the tubercle bacillus are commonly designated as *tuberculins*. The term was first employed in 1884 by Pohl Pincus.¹ Koch himself first called his old tuberculin "lymph" and it was known for some time as "Koch's lymph." Bujwid² is quoted by Reeser³ as being first to apply the term "tuberculin" to Koch's original extract and which Koch later adopted for his preparation.

Kinds of Tuberculins.—The knowledge that tubercle bacilli and their

¹ Quoted by Riviere and Morland, *Tuberculin Treatment*, Frowde, Hodder, and Stoughton, London, 1913, 31.

² *Gazeta lekarska*, 1891, No. 4.

³ *Centralbl. f. Bakteriöl.*, orig., 1908, 46, 56.

secretions as seen *in vitro* contain both desirable and undesirable substances has led Koch and others to adopt different methods of preparing tuberculin in the endeavor to obtain the desirable immunizing principles in as pure a state as possible.

As a consequence, a large number of preparations have been advocated from time to time, all of which are said to possess some special properties and virtues. All tuberculins, whatever their mode of preparation and manufacture, are derived from cultures of the tubercle bacillus. So numerous have the tuberculins become, and so superior are the advantages claimed for each new product over the older ones, both for diagnostic and for therapeutic purposes, that only a few of those possessing special interest and value can here be described.

From a practical standpoint the different tuberculins may be classified into three main groups as follows:

1. Those containing only the soluble products of the tubercle bacilli in the broth medium in which they are grown. The best known example is Koch's *old tuberculin* (O. T.).

2. Those consisting essentially of the insoluble fragments of the bacilli, as Koch's new tuberculin or *tuberculin residue* (T. R.).

3. A combination of the first two, as Koch's *bacillen emulsion* (B. E.).

The best known and most widely employed tuberculins are the following; they are usually designated by symbols:

O. T. = Koch's old or original tuberculin.

T. R. = Koch's new tuberculin; Tuberculin Rückstand or Tuberculin Residue.

B. E. = Koch's Bacillen Emulsion.

B. F. = Deny's Bouillon Filtrate.

T. B. k. = Beraneck's Tuberculin.

P. T. O. = Spengler's Perlsucht Tuberculin.

A. F. = Albumose-free old tuberculin.

Preparation of Tuberculins.—1. *Old Tuberculin* (O. T.).—This is Koch's original tuberculin, and is the variety regarded by many as the most useful both in diagnosis and in treatment. Its manufacture was based upon the principle that the toxins elaborated by the bacilli into the culture-medium or liberated by disintegration of the bodies were chiefly concerned in stimulating body cells to the formation of antibodies. Since the bacillary bodies were regarded as mainly responsible for the production of abscesses at the point of inoculation, they are eliminated by a process of filtration.

Old tuberculin is prepared as follows: Large shallow flasks containing 5 per cent. of glycerin alkaline broth are inoculated with a culture of human tubercle bacilli and grown at body temperature for from six to eight weeks, at the end of which time the bacilli have grown into a flat sheet covering the surface of the fluid (Fig. 193). The entire contents are then subjected to a current of steam over a water-bath for the purposes of sterilization and for concentration into one-tenth of the original volume. The glycerin, which is not evaporated, thus constitutes 50 per cent. of the resulting mixture. The bacilli are removed by filtration through a Berkefeld or Chamberland filter. The filtrate is a clear, brown fluid, of a characteristic odor, which keeps indefinitely and is ready for use.

Koch considered the soluble toxins of the bacillus as the desirable immunizing agents, and believed that the endotoxins were responsible for the necrotic effects. Since, however, it was accepted that bacteriolytic substances would be formed only after the injection of intact or fragmented tubercle bacilli—with their contained endotoxins—Koch added T. R. and

later B. E. to his list, in order to make the production of antibacterial substances still more complete. Furthermore, in order to obtain as varied a supply of antibodies as possible the use of several tuberculins, such as old tuberculin and bacillus emulsion, was recommended for use in the same patient.

2. *New Tuberculin (Known as T. R. or Tuberculin Residue).*—This was the next tuberculin to be promulgated by Koch,¹ and is prepared as follows: Virulent cultures of human tubercle bacilli are grown in flasks of nutrient glycerin broth for from four to six weeks, the bacilli being then filtered off and dried in a vacuum. One gram of the dried tubercle bacilli is ground in an agate mortar until all the bacilli have been broken up. To the pulverized mass 100 c.c. of distilled water are added, and the mixture is then centrifugalized. The clear supernatant fluid is poured off, and is now known as Tuberculin Oberes (T. O., not to be confounded with O. T.). It contains substances not precipitable by glycerin. The sediment is again dried, powdered, taken up in a small amount of water, centrifuged, the supernatant fluid poured off, and the process repeated until no sediment is

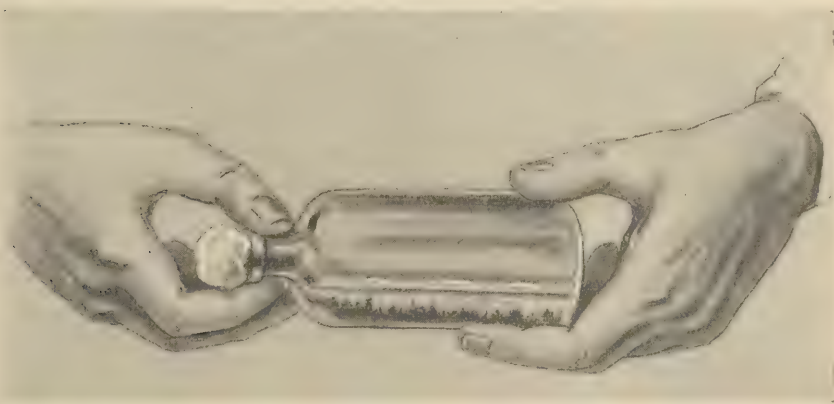


FIG. 193.—PREPARATION OF TUBERCULIN.

A flask of bouillon culture of tubercle bacilli (three to four weeks). Note the surface layer of bacilli with stalactite formations.

precipitated except that composed of gross accidental particles. The fluids resulting from all the centrifugalizations, except the very first, are poured together and the total should not measure more than 100 c.c. This opalescent fluid is preserved with 20 per cent. of glycerin and is known as T. R. It should contain in each cubic centimeter 2 milligrams of solids, representing 10 milligrams of dried tubercle bacilli.

3. *Bacillen Emulsion (B. E.).*—This was a still later form of tuberculin made by Koch,² and, as its name indicates, it is an emulsion of tubercle bacilli. The culture is grown as for O. T.; the bacilli are filtered off, ground, but not washed, and 1 part of the pulverized material emulsified in 100 parts of distilled water; an equal part of glycerin is then added, making a 50 per cent. glycerin emulsion, each cubic centimeter of which contains the immunizing substance of 5 mg. of dried tubercle bacilli. Since B. E. was not washed, it was assumed that it would retain all extractives and the entire contents of the bodies of the tubercle bacillus.

¹ Deutsch. med. Wchn., 1897, xxiii, 209.

² Deutsch. med. Wchn., 1901, xxvii, 839.

While Koch was preparing these various tuberculins others were being made, one of which, prepared by Denys, is used extensively at present in the treatment of tuberculosis.

4. *Bouillon Filtrate (B. F.)*.—This is practically Koch's old tuberculin unheated. It is prepared by Denys¹ in the same manner as O. T., except that the bacillus-free filtrate—a clear fluid said to contain only the soluble secretions of the bacilli plus the metabolized culture-medium—is not heated or concentrated and is ready for use without any further modification.

5. *Beraneck's Tuberculin*.²—This is a preparation for which its inventor claims only minimal toxicity and a high content of specific substances. It is prepared by cultivating the bacilli on a non-peptonized 5 per cent. glycerin-bouillon medium, which is not neutralized. The filtrate from this culture is known as T. B., or toxin bouillon. The residue is shaken for a long time at from 60° to 70° C. with 1 per cent. orthophosphoric acid. Equal volumes of the unheated toxin bouillon and of the acid extract of the bacillary bodies are combined to form the whole tuberculin.

6. *Spengler's Pertsucht Tuberculin (P. T. O.)*.—This tuberculin is prepared by Spengler³ with bovine bacilli from cattle suffering with pearl disease in exactly the same manner as Koch's old tuberculin employing bacilli from human lesions. Spengler believes that this tuberculin is indicated in the treatment of human beings since bovine bacilli are less virulent.

7. *Albumose-free Old Tuberculin (A. F.)*.—This tuberculin is Koch's old tuberculin from which the albumoses have been removed by precipitating with six volumes of absolute alcohol. After standing twenty-four hours the supernatant is filtered and evaporated at 56° C. over a water-bath to the original volume of O. T. employed. The fluid is now sterilized by means of candle filtration.

8. *Dixon's Tubercle-bacilli Extract*.⁴—Dixon has prepared a tuberculin by treating cultures of tubercle bacilli with ether and extracting in salt solution. This has yielded good results in the treatment of tuberculosis. This product is prepared from the living organisms. The tubercle bacilli are grown on 5 per cent. glycerin veal broth for a period of from six to eight weeks at a temperature of 37.5° C. They are removed from the incubator and collected on hard filter-paper. The filtrate of glycerin broth on which they are grown is discarded. An equal quantity, by weight, of tubercle bacilli of the bovine and human type is used. The mass of organisms is partially freed from excess of moisture by placing it between two sterile filter-papers, after which it is placed in a dish in the incubator for from twenty-four to forty-eight hours. The dried organisms are then washed in an excess of ether, which is allowed to act until it has removed all the water and glycerin. The organisms are then subjected to an excess of fresh ether and washed in this for six hours, to soften the wax of the bacilli. This fat separates so that it collects at the bottom of the vessel and is removed with a Pasteur pipet. After the second addition of ether has been removed the mass is allowed to dry until no ether odor is perceptible. After the mass of tubercle bacilli has been thoroughly dried it is ground in a mortar and suspended in physiologic salt solution in the proportion of 1 part of the ground mass to 5 parts of an 8 per cent. salt solution. This suspension is shaken in a shaking machine for from eight to ten hours, and is then allowed to stand for several days at room temperature. It is finally passed

¹ Le Bouillon Filtre de Bacille de la Tuberculose, Louvain and Paris, 1905.

² Rev. méd. de la Suisse rom., 1907, 27, 444.

³ Deutsch. med. Wchn., 1904, No. 31; *ibid.*, 1905, Nos. 31 and 34.

⁴ Penna. Health Bull., No. 28, April, 1914.

through impervious bacteria filters several times and the filtrate examined microscopically, bacteriologically, and physiologically. Culture tests are made to determine its freedom from contaminating organisms, and guinea-pigs are inoculated to ascertain that it contains no living tubercle bacilli. One cubic centimeter of this extract represents 0.2 gm. of the organisms, and is known as the stock solution, from which serial dilutions are made. This solution is sterile, but 0.5 per cent. of phenol (carbolic acid) is added as a preservative to prevent subsequent contamination.

While the tuberculins just described are those mainly used, many others have been prepared and advocated in the diagnosis and treatment of tuberculosis. The aim is always to obtain the specific substances with as little as possible of the toxic substances—not only those concerned in producing necrosis, but likewise the protein constituents responsible for specific sensitizing action and anaphylactic disturbances. For example, tuberculocidin and tuberculol are examples of attempts at isolating the pure immunizing principle; endotin, or Moeller's tuberculin, is an example of an endeavor to rid the culture fluid of protein substances.¹

The Effects of Tuberculin.—The toxicity of the different varieties of tuberculins vary in some degree, that is, the toxic effects for normal non-tuberculous human beings and lower animals due to products of the tubercle bacilli, the tubercle-proteins and various substances that may be present in the culture-medium employed for the preparation of the tuberculin. This subject will be discussed in more detail shortly; here it may be stated that old tuberculin (O. T.) is least toxic, while those composed of the bacillary fragments are apt to be somewhat more irritating or toxic due to the presence of protein substances in common with bacterial vaccines in general.

But all tuberculins are capable of producing local (at the site of injection), focal (at the site of disease), and constitutional reactions in the majority of tuberculous individuals when given in sufficient amounts, and few subjects in medicine are as perplexing as the nature and mechanism of the action of the tuberculins despite a very large amount of clinical and laboratory investigation. Brought forward by its great discoverer as an immunizing and therapeutic agent, we have since learned that its immunizing power is almost negligible and its application in treatment surrounded by definite limitations. From the very beginning when it led its discoverer grievously astray and induced him to make hasty statements and predictions, it has continued to baffle some of the best scientific minds in unraveling the nature and mechanism of its action.

In the first place tuberculin is not a poison or toxin because relatively enormous amounts may be administered to non-tuberculous animals with impunity. Kraus has injected as much as 30 c.c. intravenously to *healthy* guinea-pigs, and Hamburger has given *healthy* infants as much as 1 c.c. at close intervals repeatedly over a long period without ill effects. But the injection of one-millionth of 1 c.c. may make a tuberculous adult ill. For the tuberculous, therefore, tuberculin acts like a virulent poison; for the non-tuberculous it is relatively inert.

Why the difference? From whence arise the toxic effects in the tuberculous individual if the tuberculin is not toxic? The evidence quite clearly indicates that these toxic substances arise from the tuberculous foci, and tuberculin stimulates and facilitates their absorption by producing hyperemia and other inflammatory changes in the tissues surrounding these foci. Similar effects may be produced by the patient by exercise. The ordinary con-

¹ For a full account of these and other preparations I refer the reader to the book of Hamman and Wolman, *Tuberculin in Diagnosis and Treatment*, 1912, Appleton & Co.

stitutional symptoms of tuberculosis are quite similar to the general tuberculin reaction, and both are due to the passage of toxic substances from the focus of disease into the general circulation.

The Allergic Nature of the Tuberculin Reaction.—Why and how does tuberculin produce this inflammatory reaction around foci of tuberculous infection? This is an unsolved mystery, but evidence indicates that it is an allergic phenomenon—local allergic shock as discussed in Chapter XXXI.

The allergic nature of the tuberculin reaction has been generally held since this view was proposed by Wolff-Eisner. It is commonly believed that as a result of infection an antibody is produced in the blood of the nature of an amboceptor or ferment which acts upon the injected tuberculin with the production of a poison (anaphylatoxin) responsible for the general, focal, and local (skin) reactions. But there are few or no facts to substantiate this theory. In the first place the presence of this antibody has not been conclusively demonstrated in the blood of tuberculous individuals or lower animals. An allergic antibody is produced, but it apparently is cellular—that is, upon or within the cells of the body rather than free in the blood. Even though it were free in the blood and capable of producing a poison by digestion of tuberculin, it is impossible to conceive how sufficient poison could be obtained from a thousandth or millionth part of a cubic centimeter to produce the profound changes sometimes following injection.

Anaphylaxis is commonly regarded as caused only by protein substances. The chemical nature of tuberculin is biuret free and dialyzable; if it is a protein, evidence indicates that it is a polypeptid or proteose, and anaphylaxis of the lower animals cannot be produced regularly with proteoses. Many investigators have stated that proteoses could not induce anaphylaxis at all, but recent investigations indicate that weak reactions are sometimes observed, but granting this, attempts to produce anaphylaxis in animals with tuberculin have generally failed. Tuberculin does not appear to produce anaphylactic sensitization and reactions.

How then can the tuberculin reaction be regarded as an allergic phenomenon? The tuberculin reaction can only be produced with regularity by active tuberculosis. Living tubercle bacilli must be introduced and actual lesions result before tuberculin hypersensitiveness is produced. This suggests that allergic sensitization is engendered by a substance elaborated only by living tubercle bacilli in living tissues. Is this allergen the same tuberculin as produced in culture-media in the test-tube? Either it is the same or it is a very closely allied combination product of tuberculin and tissue cells because the tuberculin reaction is elicited by the injection of artificial tuberculin and the allergic reaction is highly specific—that is, the substance eliciting the reaction is the same or very nearly so as that engendering sensitization. If this is true, the failure to sensitize normal animals with artificial tuberculin is due either to some undefined biologic difference between focus tuberculin and artificial tuberculin, or we do not know how to sensitize animals artificially with tuberculin. The latter assumption, however, is less significant than the former because we can with comparative ease sensitize and anaphylactize guinea-pigs with proteins of the tubercle bacillus.

The allergen, therefore, is produced in the foci of tuberculosis; it gradually brings about an exquisite degree of active sensitization of the tissue cells not only surrounding the focus, but of distant organs as well, including the skin and mucous membranes. As a result of sensitization these sensitized cells contain the allergic antibody. When tuberculin is injected it unites with these sensitized cells, producing cellular allergic shock. This shock is characterized by its effect upon the vasomotor system, as is true of other

types of allergy in man, as serum disease, hay-fever, and allergic asthma. One effect is hyperemia or dilatation of the vessels accompanied by serous and cellular exudation. If tuberculin has been injected subcutaneously in sufficient amounts this reaction of hyperemia and exudation takes place around the foci of tuberculosis, and, depending upon the degree of reaction and the amount and permeability of the fibrous capsule, results in the absorption of the focus poisons with the production of general symptoms. When applied to the skin the effects are local and characterized by a local reaction of hyperemia and edema.

If the local and focal reaction to tuberculin is allergic shock and the constitutional reaction the result of increased absorption of poisons from the foci, are these reactions of therapeutic value? In the first place, what is the nature of the focus poisons? Krause has worked upon this subject extensively and believes them to be non-specific poisons produced by dead and decayed cells. These cellular substances are highly toxic and can be produced from a variety of cells and other protein substances. They are non-specific and much of the nature of Vaughan's protein poison.

The Curative Activity of Tuberculin.—Has tuberculin a curative value—that is, is the tuberculin reaction curative? Can the allergic tuberculin shock reaction redound to the benefit of the tuberculous individual? This has been and continues to be a question of vital importance, calling forth an enormous amount of clinical and laboratory investigation and a vast literature.

We have seen that the tuberculin reaction is accompanied by the production of hyperemia and serous and cellular exudation. It is commonly believed that we recover from tuberculosis by the production of fibrous tissue around the foci. The thicker and denser this capsule, the more complete is clinical recovery even though the capsule may enclose living bacilli. The cutting off of blood- and lymph- supply to the focus by this fibrous capsule gradually results in death and liquefaction of the contents, including the bacilli. Anything favoring the development of this capsule is, therefore, therapeutically advantageous.

Has the tuberculin reaction this effect? According to Krause, who has studied this problem with particular care, it has. It is a well-known principle of pathology that chronic passive congestion is followed by fibrosis. Tuberculin in proper dosage and spacing of injections can elicit a series of mild allergic shocks about the focus of disease maintaining a more or less constant degree of hyperemia. Evidence indicates that this is followed by fibrosis, and fibrosis is greatly desired. Therefore, in chronic tuberculosis where the lesions are localized in a certain organ or organs, the administration of tuberculin is advantageous. In miliary and disseminated tuberculosis it may not be, or its curative effects may be too slow to prove of benefit.

The great mistake made in the early days of tuberculin therapy was the administration of too large amounts. These large doses produced profound and injurious focal reactions with the absorption of enormous doses of focus poisons, with disastrous effects—the lamentable days of the tuberculin delirium, from the impressions of which both the profession and the laity have not yet fully recovered.

I believe that tuberculin has a very definite place in the treatment of some cases of tuberculosis and especially those of a chronic and localized character. But the doses must be small enough and so spaced that the degree of focal reaction is mild, but more or less continuous. The physician administering tuberculin should possess a good working knowledge of its effects, its potencies for harm and good; the beneficial results are not sudden or spectacular, but slow and plodding. Patience and skill are required,

and especially the patience to give a long series of subcutaneous injections, and skill in judging the correct dose. In general terms the latter is judged according to the amount of absorption of foci poisons because the hyperemia cannot be seen in the internal organs; the degree of constitutional effects should be slight, as shown by only a degree or two of febrile reaction and minor other effects. Finally, it is very difficult to express the effects by percentages of cures; individual cases in the practices of physicians may not have been benefited, but the effects of mild allergic tuberculin shocks appear to be favorable in that they aid in the production of fibrous tissue, and suitable patients should always have the possible benefits of this process.

Antibody Production from Tuberculin Treatment.—Aside from the possible curative effects of the tuberculin reaction about foci of tuberculosis from hyperemia, exudation, and fibrosis, do the various tuberculins engender antibodies possessing curative effects?

As previously discussed under Immunity in Tuberculosis antibody production in this disease does not progress very far. At least the amounts of antibodies (opsonins, agglutinins, lysins, etc.) found in the blood are usually small and almost insignificant; the injection of immune serum does not confer more than a slight degree of passive immunity, and the curative effects of antituberculosis sera are to be ascribed more to non-specific protein therapy than specific agents.

Immunization of the lower animals with the various tuberculins as well as with ordinary-heat killed and even living non-virulent vaccines is followed by slow antibody production; months and even years are usually required and the sera at best contain relatively small amounts of agglutinins, opsonins, and complement-fixing antibodies without being markedly tuberculocidal. This is especially true when old tuberculin is employed for immunization. In my experience several months are required with increasing doses every five to seven days for the production of even small amounts of complement-fixing antibodies. With bacillary antigens antibody production is somewhat more rapid, but the tubercle bacillus is but poorly antigenic, in so far as the production of serum antibodies in the guinea-pig and rabbit are concerned.

It is doubtful if antibodies are produced during the treatment of tuberculosis with tuberculins in sufficient amounts to exert curative effects.

Insusceptibility or Tolerance to Tuberculin.—It is commonly observed that tuberculous individuals develop a tolerance for tuberculin under treatment. At the beginning the subcutaneous injection of 0.0001 c.c. of old tuberculin may produce local, focal, and constitutional reactions, whereas after six months of carefully graded and spaced injections doses of 0.01 c.c. and even 0.1 c.c. may produce no reactions at all.

Formerly this increased tolerance was ascribed to the production of antibodies for the tuberculin capable of neutralizing the supposedly toxic properties of tuberculin. But it is now quite clear that tuberculin is not primarily toxic and does not engender the production of antibodies for itself and the tubercle bacillus except to a very limited degree. A more reasonable explanation which has the support of experimental and clinical data is that the tolerance is due to the progressive development of fibrous tissue about the tuberculous lesions preventing the escape of foci poisons.

Doubtless the products of the tubercle bacilli in the lesions are also capable of acting as tuberculins and producing the allergic reactions in the periphery of hyperemia and exudation; this explains the focal and constitutional reactions following any procedure unduly disturbing the foci, as exercise or work. Healing, which is fibrous encapsulation, gradually increases

the mechanical barrier to absorption of foci poisons, explaining why the tuberculous subject is able to progressively increase exercise and work without eliciting reactions.

In addition to this mechanical factor it is possible that a part of the increased tolerance to artificial and foci tuberculins is due to allergic desensitization of the tissues to these substances. In acute fulminating tuberculosis with little or no encapsulation of the lesions the well-known insusceptibility or tolerance to injections of tuberculin is probably due to a process of desensitization of the tissues by the enormous production and unrestrained distribution of foci tuberculins.

Increased Sensitiveness to Tuberculin.—While the administration of tuberculin is usually followed by a gradually increasing tolerance for larger and larger doses, just the reverse may occur. A patient may stand the injection of 0.01 c.c. of old tuberculin without demonstrable focal and constitutional reactions and yet some time later the injection of 0.001 or even 0.0001 c.c. may elicit well-marked reactions. During the tuberculin treatment of tuberculosis one may find it necessary to make a sharp drop in the dosage by reason of the occurrence of this increased sensitiveness.

A reasonable explanation is that hyperemia persists about the foci longer than expected or is greater than anticipated, and for this reason a much smaller dose of tuberculin is capable of increasing the hyperemia and absorption of the foci poisons. At one time it was supposed that allergic hypersensitiveness was produced by the injections of tuberculin which thereby added to the degree of sensitization produced by the disease, but it is now well proved that old tuberculin does not sensitize or only to a very mild and limited degree, although the administration of tuberculins containing the bacillary bodies may result in some degree of anaphylactic sensitization to tubercle proteins. But this possibility is remote and does not exist practically when old tuberculin is being administered. The purely mechanical factors of increased hyperemia of the capsule and absorption of focus products are a more acceptable explanation of increased sensitiveness to injected tuberculin and the increased susceptibility to the focus tuberculins, as a result of exercise or other factors disturbing the lesions.

Non-specific Reactions in Tuberculosis.—Not only injections of tuberculin may elicit focal and constitutional reactions in tuberculosis, but other substances as well. If the reactions are due to the escape of foci poisons this is not difficult to understand. Anything increasing hyperemia about the lesions may be expected to increase the absorption of these toxic substances. In the administration of such non-specific agents as proteoses, milk, typhoid, and various other vaccines, in amounts capable of themselves of eliciting reactions of chills, fever, and leukocytosis, etc., it is natural to expect that foci of infection regardless of the kind of organism concerned will show more marked reactions than normal and healthy tissues. Work and exercise alone apparently increase the degree of hyperemia about tuberculous foci and the absorption of the toxic focus substances, and may be regarded as non-specific stimulants.

But these observations do not question the specific action of the tuberculins which may engender local, focal, and constitutional reactions in amounts very much less than required of such non-specific agents as peptone, milk, and typhoid vaccine administered by subcutaneous injection.

Choice of Tuberculin for Treatment.—How do the large number of tuberculins compare in their effects and therapeutic value? Is there a preference for tuberculin of bovine bacilli in the treatment of some cases of tuberculosis?

In answer to the first question, all tuberculins are essentially the same in the general effects produced, although varying greatly in their potency, which alters the dose, intensity, and duration of the focal reactions.

Special claims are made for certain tuberculins, but it is the consensus of opinion that the focal reactions are the same and that more depends on the method of administration than on the kind of tuberculin. Possibly something is to be gained by changing tuberculins during the course of treatment. For example, after old tuberculin has been given for some time it may be well to use B. E. (bacillen emulsion) or some other tuberculin containing the bodies of the tubercle bacilli in addition to their extractives.

Since the active principle in tuberculin is unknown and has not been isolated in a pure state, it is difficult to compare the strengths of different tuberculins. One way is by determining the minimal amount required to kill tuberculous guinea-pigs; another is by quantitative skin tests.

Von Behring has found old tuberculin forty-two times stronger than T. R. (tuberculin residue). T. R. (tuberculin residue) and B. E. (bacillen emulsion) are generally weaker than O. T. (old tuberculin).

All tuberculins are relatively feeble in direct toxicity; certainly they do not appear to be more toxic for normal individuals than most ordinary vaccines. But some tuberculins are less toxic than others. Old tuberculin is said to contain about 10 per cent. albumoses, and the removal of these appears to reduce the toxicity in some degree (the A. F. or "albumose-free" tuberculin of the Farbwerke Höchst Company). Baraneck's tuberculin (T. Bk.) is said to be least toxic, followed in order by A. F. (albumose-free tuberculin), B. F. (Deny's bouillon filtrate), and O. T. (Koch's old tuberculin).

A good deal of difference exists among tuberculins in regard to the rate of absorption. Extract tuberculins, as O. T., B. F., and A. F., are more rapidly absorbed and produce their effects more quickly than bacillary suspensions, as B. E. The latter are less likely to produce immediate reactions, are milder in action, but more difficult to gauge in dosage.

Bovine tubercle bacilli are apparently less virulent for human beings than human tubercle bacilli and human bacilli, are less virulent for cattle than bovine bacilli. Undoubtedly tuberculosis in human beings is sometimes due to bovine infections and especially tuberculosis of the mesenteric glands of children. For these reasons some observers have advised the use of bovine tuberculin for the treatment of tuberculosis of human beings as representing a tuberculin of less virulent bacilli.

Koch, however, saw no difference in the effects of tuberculins made of human and bovine bacilli, although one or the other may be somewhat stronger depending more upon quantitative cultural differences than a vital or essential difference in the active principle of tuberculin. Other observers with extensive experience have reached the same conclusion, and for the treatment of tuberculosis of human beings tuberculin is usually made of human bacillus, and for veterinary purposes bovine bacillus is employed.

Autogenous Tuberculins.—Owing to the great amount of time and labor required for the isolation of individual strains of tubercle bacilli, only a small amount of investigation has been conducted in the treatment of tuberculosis with autogenous tubercle vaccines or tuberculins. Some observers believe that the results have been better, but all tuberculins are similar in their effects upon tuberculous lesions bringing about hyperemia, exudation, increased absorption of foci tuberculin or other toxic substances and facilitating encapsulation. If tuberculin itself has virtues in the treatment of tuberculosis aside from bringing about these allergic focal reactions, the patient probably derives more benefit from absorption of his own foci

tuberculin (autotuberculinization) than from the relatively small amounts of artificial tuberculin administered.

Indication for Tuberculin Treatment.—Tuberculin is not primarily toxic in the small amounts usually administered, and does not itself add to the symptoms of toxemia, but a sufficiently large dose may augment the toxemia by increasing the absorption of foci poisons. For this reason a distinction is to be drawn between those cases with and without auto-intoxication.

1. Patients afflicted with incipient tuberculosis are proper subjects for receiving tuberculin treatment, since it tends to protect them from open lesions and relapse, and insures, to a greater degree, their ability to continue work.

2. Advanced and moderately advanced cases may be given tuberculin if the nutrition is fair, the febrile reaction mild, the pulse not very rapid, and if the treatment is controlled by rest. Old fibroid cases with fair nutrition are especially suitable, as such patients become capable of moderate activity and are much less likely to suffer from relapses.

3. Cases of tuberculosis of the lymphatic glands, skin, and special organs may be benefited by prolonged and careful tuberculin therapy.

4. Cases of latent tuberculosis, especially the children of infected families who are below par physically and show tuberculin hypersensitiveness, with indefinite physical signs, are proper subjects for receiving tuberculin treatment.

5. The question arises as to whether tuberculin may be administered to ambulant patients. Tuberculin should be regarded as but one factor in the treatment of tuberculosis, and, as such, should be combined with the best therapeutic measures available. Therefore the tuberculin treatment is supplementary to rest, hygiene, and fresh air, and the benefit of the sanatorium should not be denied to patients, especially to the poorer ones. The treatment of a mildly progressive ambulant case should be undertaken only when prolonged rest in bed has had no visible effect, and when no measures can be devised for administering the tuberculin while the patient is in bed, as, *e. g.*, patients who have been at the sanatorium and have returned to work, or those who cannot be persuaded to enter a sanatorium or for whom no place can be found. The tuberculin treatment of more chronic or localized tuberculosis may be successfully undertaken in the clinic or office.

Contraindications to Tuberculin Therapy.—Owing to the increased focal hyperemia that follows the injection of tuberculin, hemoptysis has been considered a contraindication to its use. In such cases it is well to wait for some time at least and begin the injections with very small doses, as the ultimate effect, namely, the production of fibrous tissue, may be of great aid in prolonging life.

Various authorities have expressed different views regarding other contraindications, such as marked general weakness, fever, cardiac disease, nephritis, epilepsy, syphilis, hysteria, etc. As was stated by Hamman and Wolman, these are not contraindications, but unfortunate complications that would embarrass any form of treatment. Tuberculin may be given to any patient whose resisting powers have not been too much depressed as the result of complications. For the beginner in this form of therapy, however, it is advisable that he acquire experience by undertaking the treatment of uncomplicated cases before assuming the responsibility of treating the more difficult ones.

The fact that the ophthalmic test has been made is no contraindication to treatment by tuberculin if the reaction has subsided, since a flare-up rarely occurs except after large diagnostic doses (Hamman and Wolman).

ADMINISTRATION OF TUBERCULIN

Tuberculin Reactions.—Of most importance in this connection is the attitude of the therapist toward the question of reactions following the administration of tuberculin. He must know whether he does or does not wish to obtain symptoms of a tuberculin reaction during the treatment; the size of the initial and particularly of subsequent doses will depend upon his desire to obtain a reaction or upon his anxiety to avoid it.

At the present time tuberculin is never used for the purpose of obtaining strong reactions, such as Koch originally insisted upon getting. Koch administered a dose large enough to elicit a strong constitutional reaction, and repeated it at intervals of one or more days until that dose no longer produced a reaction, after which a still larger dose was given and the former procedure repeated. Many—too many—were unable to pass through this therapeutic furnace unsigned, and, in fact, the results obtained led to the period known as the “tuberculin delirium,” ending, as Hamman and Wolman stated, “to the consequent downfall of the arrogant therapy to an humble position, whence it is but just emerging, chastened and refined, to assert its modest but now truthful claims to a therapy less spectacular, but more healing, less forceful, but more gently persuasive: healing a few, helping many, and hurting none.”

While there is this general unanimity of opinion regarding the harmful effects of strong reactions, yet tuberculin therapists may be divided into those who scrupulously avoid all reaction, those who are a little bolder and do not object to a very mild reaction, and to an intermediate class. In the first group are Sahli, Denys, and Trudeau; the former claims that it is essential, first of all, to do no harm, and that cases treated cautiously attain a tolerance for high doses as soon as, and even sooner than, those that are rushed. Petruschky, Spengler, Löwenstein, Möller, Pickert, White, and others are exponents of the bolder method, believing that, by proceeding very cautiously, much time is wasted and not enough focal reaction is produced to promote healing. To the intermediate class, and approaching rather the timid class, are Bandelier and Roepke, Hamman and Wolman, M. S. Cohen, and many others. These observers adopt no scheme of fixed dosage, but study the individual patient, remembering what is to be avoided, rather than the high dose to be reached.

The *constitutional symptoms of a reaction* are: temperature, loss of weight, rapid pulse, and general symptoms, such as malaise, headache, chilliness, arthritic pains, gastric or intestinal disturbances, nausea, loss of appetite, insomnia, and skin eruptions.

Of these general signs, the most important are fever, loss of weight, and symptoms of general depression. The temperature should be taken for several days preceding the initial dose, so that the patient's “normal” limits are known before the tuberculin is given. When the usual maximum temperature is reached, it is especially necessary to watch closely for additional signs of reaction. Without some constitutional disturbance a slight pyrexia is of less significance, and it is to be remembered that tuberculous patients may have a flare-up of fever when they are not being treated with tuberculin. Denys refuses to consider any temperature reaction as due to tuberculin that comes on more than forty-eight hours after the injection has been given. It is characteristic of tuberculin reactions that the rise is abrupt, and not in step-like progression. Hamman and Wolman would hesitate to ascribe an elevation coming on suddenly in the midst of a perfectly smooth course of tuberculin treatment, and unaccompanied by a local reaction to the injections, when the dose has not been unduly large.

Loss of weight is a delicate sign—more valuable as a symptom of overdosage late in the treatment than as a protection against the suddenly appearing reactions.

Bandelier and Roepke regard an increase in the pulse-rate as a sign of great importance. Hamman and Wolman and Lawrason Brown have not been able to observe this sign very frequently.

The *local signs* are: pain, tenderness, and swelling at the site of injection. These may consist of all gradations from simple thickening of the skin to a wide, deep, hard, and painful node, with or without involvement of the neighboring lymphatics.

The local reaction has assumed great importance in recent years, especially since Denys drew attention to it as serving as a warning of the approach of a general reaction. It is characterized by the development of pain, tenderness, and redness about the site of a former injection. It is more often solitary than any other sign, and, in the absence of a temperature record, it is safe to proceed, the sole guidance being the local reaction, both subjective and objective. A large dose of tuberculin may give a local reaction, due merely to its bulk and concentration (500 to 600 mg.), simulating a true reaction; this may be avoided by dividing the dose into two injections, which are given at the same time, but not into neighboring areas of skin.

The *focal signs* in pulmonary tuberculosis are: increased cough, dyspnea, expectoration, thoracic pain, hemoptysis, and extension of the physical signs.

Focal signs of a reaction are an indication that the treatment has been conducted too rapidly.

The Favorable and Desirable Tuberculin Reaction.—Unless one belongs to the ultraconservative class of tuberculin therapeutists, it is not a slight focal reaction that is to be avoided, but those reactions that are large enough to manifest themselves by changes in the physical signs or by decided symptoms. On the contrary, it is the production of such slight hyperemia about the focus of disease that constitutes the most valuable result of tuberculin therapy. It is well to start with a minute dose, and push the dosage rapidly until a slight focal reaction at the site of injection or mild pyrexia is observed. When a mild focal reaction is not produced, the patient is not receiving his due amount.

As stated by Cohen¹ success in tuberculin treatment can be expected only when the individual patient is given his appropriate dose, whether by chance, accident, in the course of systematic gradual increases, or by first determining this dose by intracutaneous tests.

As stated above, I believe that it is desirable to produce mild focal reactions, but more importantly, a *favorable constitutional reaction* defined by Cohen² as an improvement in appetite, increase of strength, a feeling of well being; lessening of symptoms, flattening of the temperature curve, and all favorable phenomena occurring on the day tuberculin is given or on the following day.

The Choice of Tuberculin and the First Dose.—As previously stated, all tuberculins are essentially the same in their action, but the rate of absorption and consequently the rapidity of action varies among them. The extract tuberculins, like O. T., are more quickly absorbed than the bacillary suspensions or vaccines, as B. E. and T. R. My own preference is for O. T., Koch's old or original tuberculin; for febrile patients where slower absorp-

¹ Arch. Pediat., November, 1920.

² Jour. Amer. Med. Assoc., 1914, 63, 1386; Med. Record, 1914, 86, 756.

tion may be desirable, I use T. R. (tuberculin rückstand) or B. E. (bacillen emulsion). *Whatever tuberculin is employed the principles governing its administration are the same, and the technic described below is adapted for the administration of any tuberculin.*

These directions are for subcutaneous injections; other routes of administration may be employed, to be described later.

The directions for administration are likewise adaptable for tuberculosis of different organs, but more especially for pulmonary disease.

It is very important not to give an excessive dose at any time including the first dose. Since each patient is a law unto himself, the initial dose should be so small that no harm can result from its use. Children and adults who exhibit a slight fever or are not in good condition require smaller doses than those patients in good condition.

The proper dose is that capable of eliciting a mild allergic reaction about the tuberculous foci. One may either start with an arbitrarily small dose and rather rapidly increase it until a favorable reaction is secured, or one may first conduct skin tests and determine by this means the sensitiveness of the patient. The latter is preferable, but laborious and not essential for success.

In the arbitrary method I believe that *an initial dose of any tuberculin may be 0.0001 c.c. for adults by subcutaneous injection.* In cases of marked auto-intoxication with fever of over 100° F. and tachycardia, the dose may be 0.00001 c.c. Children under twelve years require less according to body weight.

White and von Norman¹ use a cutaneous or von Pirquet test with 0.0001 c.c. of O. T. (never with T. R.) and reduce or increase this amount until they produce a minimal reaction, namely, one that shows redness and swelling measuring 4 to 6 millimeters in diameter within seventy-two hours. They then inject intracutaneously the exact amount that produced this reaction every two weeks for a period of three months. Another test is made at this time and treatment continued as heretofore.

My own method is slightly modified after that employed by Cohen,² who gives a series of intracutaneous injections using the skin of the forearm. At the first time four injections are made at least 1½ inches apart as follows:

0.1 c.c. of 1 : 1,000,000 (0.0000001 c.c.) below the elbow.

0.1 c.c. of 1 : 100,000 (0.000001 c.c.) next below.

0.1 c.c. of 1 : 10,000 (0.00001 c.c.) next below.

0.1 c.c. of 1 : 1000 (0.0001 c.c.) above the wrist.

The method of preparing these dilutions is described below. The unit of measure of tuberculin is commonly expressed in milligrams (mg.), but inasmuch as all tuberculins are fluid and not solid, the measure should be in fractions of the cubic centimeter or c.c. (milliliter or ml. is now being used by many instead of cubic centimeter or c.c.).

The reactions are read forty-eight hours later and a papule and induration regarded as positive. If no reactions occur, a second series of injections are given in the skin of the opposite arm using these doses:

0.1 of 1 : 100 (0.001 c.c.) below the elbow.

0.1 of 1 : 10 (0.01 c.c.) next below.

In the majority of instances, however, the first set of injections shows the smallest amount of tuberculin giving a distinct reaction and this amount is the first dose to be given by subcutaneous injection.

¹ Jour. Med. Res., 1909, 20, 347; *ibid.*, 1909, 21, 225; Arch. Int. Med., 1910, 6, 449; *ibid.*, 1912, 9, 114.

² Jour. Infect. Dis., 1917, 20, 223; New York Med. Jour., 1913, 98, 268.

Subsequent Doses and Intervals Between Injections.—The interval between injections is usually from three to four days—*i. e.*, two injections a week. One of these injections should be given on Saturday, in order that the patient, if ambulant, may have the advantage of rest over Sunday. This interval is merely tentative, and while it should not be shortened, it may be necessary to prolong it. While most reactions set in within from twenty-four to thirty-six hours, some may begin as late as from forty-eight to sixty hours (L. Brown). By waiting at least three days we may be assured that, if no reaction has occurred, none will take place.

The usual interval is maintained as long as the patient is doing well. After a while the patient becomes intolerant and exhibits slight reactions, depression, and loss of weight. In such instances the interval may be increased to a week and the injections continued. Hamman and Wolman find this occurrence so frequent that they advise increasing the dose interval to one week, when the dose of 0.1 c.c. of O. T. is reached, 0.2 c.c. of T. R. and B. E., and 50 mg. of B. F.

The aim should be to avoid the unfavorable reactions and gain the favorable. Both physician and patient should be thoroughly familiar with these. If any dose produces an unfavorable reaction the next dose should not be given until this subsides and should then be not more than one-half or even less than the former dose depending upon the degree of reaction.

Practice among the most experienced phthisiotherapists varies greatly in regard to the size of subsequent injections. Probably the majority follow an arbitrary plan of beginning with a small dose and increasing upward in a gradual manner, dropping back when unfavorable reactions occur.

Cohen aims to give the dose that produces a favorable reaction and continues that dose unchanged as long as the patient continues to do well; when progress becomes stationary he increases the dose accordingly. This appears to be a sensible and safe plan.

Method of Preparing Dilutions and Doses.—In sanatoria this is a simple matter, but for the general practitioner treating a few cases may prove a troublesome feature. The technic, however, is simple, but *the dilutions should be prepared at least once a month and preferably once every two weeks*, in order to guard against employing an inert product. The tuberculin dilutions should be kept in a cold place. The stock tuberculins are prepared by various manufacturers of biologic products and are less subject to deterioration than the dilutions. For the method described below the stock tuberculin may be purchased in ampules of 1 or 5 c.c. and diluted as required.

If purchased already diluted and ready for use due care must be exercised to use a sufficiently fresh product.

The initial dose given arbitrarily may be 0.0001 c.c. as stated above; this is also the largest amount given intracutaneously, if the skin tests are conducted for determining the dose. This is equivalent to 0.1 c.c. of a 1 : 1000 dilution.

1. One should have on hand dark glass bottles, holding at least 10 c.c. and furnished with rubber-dam caps. These are sterilized in boiling water just before use. Also 100 c.c. of sterile physiologic salt solution to which 0.3 c.c. of pure fluid phenol or tricresol is added and well shaken (= 0.3 per cent.).

2. With a sterile pipet place 9.9 c.c. of the phenolized saline solution in a bottle and add 0.1 c.c. of the pure tuberculin; mix well. This gives a dilution of 1 : 100, of which 0.1 c.c. carries 0.001 c.c. tuberculin. Label, 1 : 100.

3. In a second bottle place 9 c.c. of phenolized saline and 1 c.c. of the

1 : 100 dilution. This gives a 1 : 1000 dilution, of which 0.1 c.c. carries 0.0001 c.c., the usual initial dose. Label, 1 : 1000.

4. For the skin tests or in case a smaller initial dose is to be given, higher dilutions may be prepared. In a third bottle place 9 c.c. of the phenolized saline and add 1 c.c. of the 1 : 1000 dilution. This gives a 1 : 10,000 dilution, of which 0.1 c.c. carries 0.00001 c.c. The next higher dilution prepared in the same manner is 1 : 100,000, of which 0.1 c.c. carries 0.000001 c.c.

5. As previously stated, treatment is usually begun with 0.1 c.c. of 1 : 1000 dilution representing 0.0001 c.c. of tuberculin. The next dose may be 0.2 c.c. and each succeeding dose increased (unless contraindicated) by 0.1 c.c. until a dose of 1 c.c. of this dilution has been given.

The next dose would be 0.2 c.c. of the 1 : 100 dilution and increased by 0.1 c.c. each time until a dose of 1 c.c. is reached.

This would be followed by a dose of 0.2 c.c. of 1 : 10 dilution prepared by diluting 1 c.c. of the tuberculin with 9 c.c. of the phenolized saline solution. The last dose would be 1 c.c., which carries 0.1 c.c. of tuberculin.

If still larger doses were required the injections may be continued by using 0.2 c.c. of undiluted tuberculin followed by increases of 0.1 c.c. until a dose of 1 c.c. undiluted tuberculin was being given.

As a general rule, the succeeding doses of a tuberculin may be increased by 0.1 c.c. as previously stated, due care being exercised when the dilutions are changed. If the patient is quite sensitive, it may be well to repeat the first dose of each stronger solution once or more often as it is reached, and, instead of proceeding to 0.2 c.c., give only 0.15 c.c., thus tiding over the gap. If the patient is not so sensitive, a few leaps may be taken with the weaker dilution, so as to test the patient's tolerance, and, if it is good, the next higher dilution is given at once.

Beraneck's tuberculin is marketed, ready diluted, in a series of syringes, A/128, A/64, A/32, to A/4, A/2, A, B, C, to H. H is the pure tuberculin. Each solution is one-half the strength of the next stronger one. The increase of dosage is usually by 0.1 c.c. until 0.5 c.c. is given. Then 0.1 c.c. of the next stronger dilution is given, and so on.

Dixon's tuberculin, employed by the Pennsylvania Health Department, is supplied in syringes in the series of dilutions given in the following table, so that the doses may be increased as is found desirable:

ADMINISTRATION OF DIXON'S TUBERCULIN

DILUTION No.	AMOUNT (IN GRAMS) OF EXTRACT OF TUBERCLE BACILLI CONTAINED IN	DILUTION No.	AMOUNT (IN GRAMS) OF EXTRACT OF TUBERCLE BACILLI CONTAINED IN
1.....	0.000001	9.....	0.00008
2.....	0.00001	10.....	0.00009
3.....	0.00002	11.....	0.00010
4.....	0.00003	12.....	0.00011
5.....	0.00004	13.....	0.00012
6.....	0.00005	14.....	0.00013
7.....	0.00006	15.....	0.00014
8.....	0.00007	16.....	0.00015

It is suggested that in ordinary cases the injection of each dilution be repeated at least five times before changing to the next number or next stronger dilution. Larger doses may be given by using two injections of No. 16.

Site of Injection.—Injections are probably best given in the back, at the lower angle of the scapula. Local reactions are more reliable here than when the injections are given in the arm, although injections at about the insertion of the deltoid muscle are quite satisfactory. All injections should

be given under aseptic precautions and with a sterilized syringe in exactly the same manner as any bacterial vaccine is given. All injections should be subcutaneous; intramuscular injections are to be avoided, and no great advantage is to be gained from using intravenous injections.

Time of Injection.—There is some difference of opinion as to the best time of the day for administering tuberculin therapeutically. If given in the morning, a slight febrile reaction may occur during the evening which would otherwise be overlooked. Brown is in favor of the afternoon as the most suitable time, because it affords an opportunity for omitting the dose in case there is an accidental rise of temperature on that day. On the other hand, it is contended that the rest at night would tend to prevent the occurrence of the reactions that might appear if the patient were up and about.

While it is not essential that the patient rest for a few hours after a dose has been administered, this is advisable, and where absolute rest can be enforced, the dosage may be increased with greater rapidity than in ambulant patients.

The Duration of Treatment.—The length of the course of tuberculin treatment varies considerably according to individual circumstances. The first three months are the most important and require the closest co-operation between physician and patient because of the difficulties that may occur.

The administrations should continue until the patient can stand without reaction a dose of at least one hundred times larger than the first reacting dose. For example, if the first dose were 0.001 c.c. the injections should continue until a dose of at least 0.1 c.c. has been reached. Riviere and Morland state that injections of any tuberculin may stop at 0.1 c.c. except in advanced cases where symptoms have not improved up to this dose and when the patient cannot be kept under observation. It is never necessary to exceed a dose of 1 c.c. undiluted tuberculin, although this is the custom of some, as Götsch and Schlossmann.

Some physicians, as Jochmann, employ the skin tests as a guide and continue treatment until the v. Pirquet cutaneous test yields a negative reaction. This practice is based upon the assumption that desensitization of the skin occurs as tolerance is increased, but it would appear that tolerance may be built up to a high degree by progressive encapsulation of the focus of tuberculous disease without a commensurate degree of desensitization of the skin.

It is a safe rule to continue the injections as long as the symptoms continue to improve, and the time varies from three months to one or two years. Even after sputum ceases and tubercle bacilli are no longer found, it is well to continue for a few months after this gain.

Repetition of Tuberculin Treatment.—After treatment ceases sensitiveness to tuberculin continues to decrease as tolerance increases in the favorable cases. Sensitiveness to tuberculin, however, may remain and even increase without recrudescence of the disease, due to the absorption of sufficient tubercle products to maintain and increase allergic sensitiveness of the skin and other tissues. Furthermore, the disease may suddenly spring into activity and prove destructive without an apparent return or increase of sensitiveness; this condition may be due to accidental rupture of a gland or other focus and hypersensitiveness is lost during acute infections.

However, a return of sensitiveness is usually a sign of activity of the disease and Petruschky states that this occurs in about three months and that the series of injections should be recommenced. The return of sputum or the reappearance of tubercle bacilli, cough, or any symptoms which had disappeared, should suggest a repetition of the series of injections. As long

as the patient is gaining in weight and conditions are generally favorable and satisfactory there is no indication for repetition, but upon the first evidences of backsliding the course should be recommenced.

Other Routes for the Administration of Tuberculin.—*Oral Route.*—It has been shown that reactions may follow the oral administration of tuberculin. But absorption is so irregular that a quantity of tuberculin may be absorbed suddenly and cause unexpected reactions. Much depends, apparently, upon the state of digestion and upon the condition of the alimentary tract. The oral route also deprives the physician of the benefits to be obtained from using the local reaction as a guide. Otherwise the method is simple and the tuberculin may be administered in the form of tablets or in capsules. It is important, however, to exercise supervision over the patient. S. Solis Cohen¹ has used a modification of Latham's method, and reports favorable results: "Tuberculin residue (T. R.) triturated with milk-sugar is given with skimmed milk, whey, or beef-juice. The initial dose is 0.000001 mg. Both subjective and objective symptoms of reaction are watched for. The dose is repeated once or twice weekly, according to results. It is gradually increased by increments of 0.000001 mg. to the reaction point, and then dropped one point lower, and so continued for some weeks. Later, a further increase is attempted, and if reaction is not shown, is proceeded with in a similar gradual way. The arbitrary increment of 0.000001 mg. is maintained during this remittent progression until 0.0001 mg. has been reached. After that the increment may be raised to 0.00001 mg. Thus, by successive stages, a maximum dose is attained at a point determined for each individual by all the factors in the case, including the rapidity of increase, character and intensity of reaction, and maintenance of tolerance, as well as the focal and general signs of improvement. The treatment is continued with intermissions for many months, and may be resumed, if necessary, from time to time over a period of years."

Tuberculin has also been administered *intrabronchially* and by the rectum without good results.

Koch was the first to administer tuberculin *intravenously*, but this route has not come into general favor, owing to the fact that even greater control over dosage is necessary; there is, besides, no local reaction to serve as a guide, and technically the administration is more difficult than is subcutaneous injection.

The Intrafocal Route.—The use of tuberculin intrafocally, that is, a method by which the tuberculin is brought into immediate contact with the diseased area, has been advocated in the treatment of tuberculous pleurisy, tuberculous peritonitis, and tuberculosis of other serous membranes, such as those lining joints, the tunica vaginalis of the testicle, etc. Senger, Crocker, and Pernet advise the intrafocal use of tuberculin in the treatment of lupus; others have applied it directly to broken-down glands and to sinuses. William Egbert Robertson² has reported very good results in 2 cases of tuberculous pleurisy as a result of withdrawing a small amount of fluid and injecting 5 mg. of old tuberculin through the needle, which is left *in situ* for that purpose. After some hours there was a slight reaction; the remainder of the fluid was rapidly absorbed, and convalescence was promptly established, though, of course, a damaged lung remained. In a case of hydrocele the injection of old tuberculin was followed by a sharp local and a moderate general reaction, followed by absorption, and without the slightest evidence of recurrence to date, now about a year since the injection was made.

¹ Amer. Jour. Med. Sci., 1915, 149, 81.

² Personal communication.

TUBERCULIN TREATMENT OF PULMONARY TUBERCULOSIS

Ambulant Treatment.—The tuberculin treatment of ambulant cases of pulmonary tuberculosis is becoming more prevalent. These cases may, or may not, be able to work, but come from their homes to the physician's office or to some institution at regular intervals for observation and treatment. Experience has shown that the practice is without danger and the administrations may be carefully controlled by instructing the patient to take the temperature and observe symptoms. Elaborate and very satisfactory charts have been devised for recording the progress of individual cases and a great deal can be done toward securing the confidence of the patient and his close co-operation, which are so essential for the successful treatment of this disease.

A distinct drawback to ambulant treatment is the danger of mixed infection due to the inhalation of dust and dirt. Whenever the disease is both open and recent, every effort should be made to place the patient in a sanatorium where the air is pure and better hygienic treatment procurable, until the lesion has again become closed.

A second drawback to ambulant treatment is that rest cannot be prescribed after the injections as well as in institutions. But much can be done in this direction by giving the injections late in the afternoons and particularly on Saturdays. Of course, in febrile cases where more or less continuous rest is essential, ambulant treatment should not be given unless for symptomatic relief.

Ambulant treatment, therefore, is safe for closed cases when prolonged rest in bed is not required. Also for cases returned home from sanatoria when the course of tuberculin treatment has not been completed. It is the next best thing for those who are unable to secure sanatorium treatment for social or family reasons (makeshift ambulant treatment). Distinct advantages are that the patient may remain at home and probably at work, free of financial anxiety, strange surroundings, and home-sickness.

Treatment of Children.—Tuberculosis in infants is highly fatal and the morbidity and mortality keep closely parallel during the first year of life. The glands are usually first involved and, if superficial with softening, recovery may take place; if the mesenteric and mediastinal group are involved the disease usually extends to neighboring organs and tuberculin offers little or nothing in the way of treatment. The resistance of very young children is very low with but slight tendency for encapsulation of the lesions. Tuberculin may be administered, but the chances for success are remote.

In children of school age the glands are usually involved and the tuberculin treatment of these will be shortly considered. In hilum phthisis and apical infections resistance is more marked and something may be gained by the administration of tuberculin.

The method of administration is exactly as previously described, except that the initial dose must be smaller. An excellent plan is to determine the proper initial dose by the intracutaneous tests. If these cannot be made, an arbitrary initial dose for children three to twelve years of age may be 0.2 c.c. of 1 : 10,000 dilution of O. T., T. R., or B. E.; this amount corresponds to 0.00002 c.c. which is one-fifth the usual initial dose for adults.

Treatment During Pregnancy.—It is common clinical experience to observe an increased sense of well being and a loss of some of the symptoms of pulmonary tuberculosis during pregnancy; on the other hand, a minority of cases become steadily worse. Petruschky was the first to advise tuberculin in the treatment of pulmonary tuberculosis in pregnancy; there appears

to be no contraindication to its careful administration, and Riviere and Morland state "there appears very good ground for a thorough trial of tuberculin during pregnancy in women with active tubercle, or in whom a lesion has recently been active and fears are entertained for the effect of labor."

Treatment of Mixed Infections.—True mixed infections are those in which other bacteria in addition to the tubercle bacilli have gained access to the tissues and are producing pathologic changes. Other bacteria are always to be found in the sputum of all cases, but this does not necessarily mean mixed infection.

Various organisms may produce mixed infections in pulmonary tuberculosis, the most important being in the order named; streptococci, *Bacillus mucosus capsulatus* (B. friedländer), pneumococci when abundant, staphylococci of the aureus variety and *Micrococcus catarrhalis*.

The diagnosis of mixed infection is made when it is apparent from physical signs that a cavity exists in communication with a bronchus; also when there is a hectic type of temperature, a history of secondary infection and particularly influenza or grip, and when the washed sputum shows the pyogenic bacteria in intimate admixture with tubercle bacilli.

Prophylaxis of mixed infection is of the utmost importance. The patient with an open lesion should be scrupulously guarded against colds and the like; also against dust and dirt in so far as this is possible, and best by sojourning in the country, and especially in sanatoria, where the air is purer than in cities and towns. Every effort of this kind should be made to guard against mixed infection.

In the treatment of mixed infections autogenous vaccines may prove very helpful in addition to the usual hygienic measures. Cultures of the sputum should be made with particular attention to the isolation of streptococci and pneumococci. If streptococci greatly predominate in direct smears of the sputum, they alone should be employed. The vaccine may contain 1,000,000,000 per cubic centimeter.

Extra care must be exercised in the dosage. If tuberculin is given, the doses should be small enough to produce none or but the very slightest focal reaction. The first dose of the vaccine may be 0.1 c.c. by subcutaneous injection. It is my custom to give one dose of vaccine and one of tuberculin per week (vaccine on Tuesday and tuberculin on Saturday). The doses of each are cautiously increased from week to week, as possible, until a dose of 1 c.c. of vaccine is reached. I never give more than ten doses of any one vaccine, finding that better results are to be obtained by preparing fresh vaccines from time to time, not only because they possess better vaccinogenic activities, but likewise because of the chances for changes in the bacterial flora of the lesions.

Results of Tuberculin Therapy.—The early and disastrous results obtained with tuberculin in various types of tuberculosis cannot be used at the present day as a measure for determining the therapeutic value of tuberculin.

So much depends upon the individual case, the duration and activity of the disease, the possibility of supplementing the active immunization with sanatorium treatment, the skill and patience of the physician, etc., that, from a prognostic standpoint, every case must be judged upon its own merits. The results of the modern use of tuberculin show quite clearly that it is not a "cure" for tuberculosis, but, rather, a rational and useful therapeutic aid, the best results being secured when the treatment is carried out in special institutions or by specially trained physicians who have a

practical knowledge of the difficulties, dangers, and possibilities of tuberculin therapy.

The value of this or of any therapy can be judged according to various standards: (1) Working ability; (2) duration of life; (3) the presence of tubercle bacilli in the sputum; (4) physical signs, and (5) the symptoms.

The first three are especially valuable; duration of life is, after all, the most important criterion, as anything that prolongs life is, of course, welcomed.

Kremser chose 110 patients expectorating tubercle bacilli and treated 55 unselected cases with tuberculin; of these, 22, or 40 per cent., lost the bacilli from their sputum; of those treated without tuberculin only 16, or 29 per cent., lost their bacilli. Phillippi found that 58 per cent. of his second stage cases were rid of bacilli in the sputum under tuberculin treatment, as against 19 per cent. without. Brown reports from Saranac Lake that, in the incipient class, 67 per cent. of the tuberculin patients were rid of bacilli; of the others, 64 per cent. In the moderately advanced the figures are respectively 44 and 24 per cent. Bandelier gives the reports of 500 cases, of whom 202 had tubercle bacilli in the sputum. In the following table he compares the working capacity and sputum examinations of these patients under tuberculin treatment:

	TOTAL.	STAGE I, Per cent.	STAGE II, Per cent.	STAGE III, Per cent.
Complete earning capacity on discharge.	500 cases (69.8 per cent.)	90.4	80.7	32.8
Sputum changed from positive to negative.....	202 cases (63.9 per cent.)	100.0	87.3	44.0

The parallelism between the bacillary content of the sputum and the working capacity is close and shows the value, from a statistical point of view, of sputum examinations.

Healing with and without tuberculin is qualitatively the same, although, in the opinion of Ziegler, Petruschky and Rohmer, Pearson and Gillilan, Jurgens, Neumann, and others, quantitatively the results are different, all authors agreeing on the presence of more fibrosis about the lesions than is usual in the untreated cases. Autopsy findings necessarily point with less favor to tuberculin therapy than do clinical facts bearing on the rapidity and permanency with which a lesion heals.

The influence of tuberculin cannot at present be judged from the presence of antibodies in the serum, as data bearing upon the presence or absence of antitoxins, opsonins, agglutinins, and bacteriolysins are insufficient.

General symptoms and signs, such as fever, cough, loss of weight, accelerated pulse, digestive disturbances, dyspnea, and pain, if they are due to tuberculosis, as a rule improve under tuberculin treatment. Under proper conditions the incidence of hemoptysis is not usually increased, and the quantity of sputum and number of expectorated bacilli gradually decrease.

Riviere and Morland have stated the following in reference to the value of tuberculin in the treatment of pulmonary tuberculosis:

"Certain results may be said to be well established by clinical experience. The first and most striking of these is that phthisis treated with tuberculin before it has become open—*i. e.*, before it has been exposed to the risk of secondary infection—remains closed. The importance of this fact, on which

there is practically unanimous opinion, can hardly be exaggerated. It is true that the same result has been claimed for hygienic treatment. Bandler regards the fact as being so well established that he refrains from giving tuberculin to these patients because it is unnecessary. It is also true that the *vis medicatrix naturæ* unfettered by art would have had the same result in a large proportion of cases—the Paris Morgue (quoted by Huggard) gives 68 per cent. of cures without the bias of any pet remedy; but there remains a proportion, it may be small, of closed pulmonary tuberculosis which will not get well, and with these tuberculin has been shown to be competent to deal. Early diagnosis—that is to say, *really* early diagnosis, before tubercle bacilli appear in the sputum—combined with specific treatment insures completely against a breakdown.

"We believe that statistics have already shown the ability of tuberculin to increase the percentage of those who lose their sputum, or the tubercle bacilli contained in it, during hygienic treatment, and to extend the expectation of working efficiency after hygienic treatment; but we are content to leave this to a more rigid demonstration. Of all these matters the tubercular patient is the final judge, and misled as he was by the disasters of 1890-91 there is no doubt that his experience of tuberculin under the new conditions is making him willing, and sometimes even anxious, to submit himself to treatment with the remedy."

TREATMENT OF TUBERCULOUS ADENITIS

These infections usually occur in childhood and may involve the superficial glands of the neck (scrofula) or the deeper glands, as those in the mediastinum and mesentery.

In enlarged but not caseous glands tuberculin treatment may be of some assistance and aid in bringing about resolution. When softening has occurred tuberculin is of no aid in the absorption of the caseous material, surgical removal of the gland or group of glands followed by tuberculin treatment being the method of choice. When sinuses exist tuberculin may prove of distinct aid in treatment if surgical measures are refused or impossible. These cases are usually mixed infections, the pus showing the presence of staphylococci, diphtheroid bacilli, and the like.

The doses should be such as to maintain a state of progressive healing or bring about a mild focal reaction of slightly increased discharge with not more than a rise of 1° F. in temperature. As a general rule injections should be given once in seven to ten days. If a vaccine of the secondary pyogenic bacteria is employed, it should be autogenous and given every ten to twelve days alternating with the injections of tuberculin. Under this plan the patient is inoculated every five or six days.

The initial dose of tuberculin for the treatment of localized tuberculosis of children five to fifteen years of age may be 0.0002 c.c., which is larger than usually given in pulmonary tuberculosis. This amount corresponds to 0.2 c.c. of a 1 : 1000 dilution. The doses are gradually increased as described in the treatment of pulmonary tuberculosis. My own practice is not to increase the dose as long as there are evidences of healing; when the condition becomes especially sluggish and at a standstill the doses of both tuberculin and vaccine are cautiously increased.

The duration of treatment is necessarily prolonged, being a matter of several months and even a year or more. Even after complete healing has become apparent, it is well to continue the injections of tuberculin for several months beyond this point.

It is to be carefully remembered, however, that tuberculin and vaccine therapy have little to offer as long as a sinus is maintained by necrotic bone; ordinary medical and surgical treatment should not be neglected as is sometimes the tendency when biologic therapy is being employed.

TREATMENT OF TUBERCULOSIS OF BONES AND JOINTS

Tuberculous dactylitis of children, tuberculosis of the spinal vertebra (Pott's disease), and tuberculosis of the hip and other large joints are the most familiar examples of tuberculous infections of these tissues. When extensive destruction has occurred, healing is a very prolonged and tedious process. Tuberculin may prove of some aid and when properly given can do no harm. In early cases of dactylitis the results are frequently very good, but in the usual case both patient and physician must expect prolonged treatment and slow progress.

In addition to placing the patient under the best hygienic conditions obtainable, fixing the part in order to reduce motion and auto-intoxication to a minimum, aspirating pus and cleaning up necrotic tissues as far as possible, tuberculin may be administered at intervals of five to seven days in gradually increasing doses to secure very mild focal reactions. The initial dose varies greatly with different patients, but for children may be 0.2 c.c. of a 1 : 1000 dilution (0.0002 c.c.) and for adults 0.5 c.c. of a 1 : 1000 dilution (0.0005 c.c.). These amounts are not likely to produce focal reactions (slight increase of discharge and slight increased pain and tenderness), but only the mildest of reactions are desirable, and if healing is occurring under one dose it should be continued unchanged as long as progress is satisfactory. Sometimes hypersensitiveness develops, in which case the dose must be decreased.

In mixed infections an autogenous vaccine may be of aid. It may be prepared to contain 1,000,000,000 per cubic centimeter, the initial dose being 0.1 c.c. followed by gradually increasing amounts. The vaccine and tuberculin may be given alternately every ten to twelve days, which means an injection every five or six days.

TREATMENT OF TUBERCULOSIS OF THE SKIN

Tuberculosis of the skin usually occurs as *lupus vulgaris*, but also as warty or *tuberculosis verrucosa cutis*, *scrofuloderma*, or tuberculosis of the skin around discharging tuberculous lymph-glands and sinuses of other tissues, and *tuberculosis cutis orificialis* or tuberculous ulcers of the skin around orifices (anal, vulvar, nasal, and oral).

Tuberculin has been rather extensively used in former years in treatment but with negative or indifferent results. At the present time, however, some dermatologists are securing good results and especially with T. R. and B. E.

The doses should be sufficiently large to produce mild focal reactions of increased hyperemia. Rather large amounts may be required for this result, and for this reason the patient should be carefully examined for pulmonary lesions which may preclude the injection of amounts sufficient for producing these focal reactions. There should be no disturbance of the general health, which should receive attention in the way of fresh air, good food, and other hygienic conditions. The initial dose of T. R. or B. E. for adults may be 0.5 c.c. of a 1 : 1000 dilution, subsequent doses being raised to secure very mild focal reactions as previously described under the administration of tuberculin.

The injections may be given every seven to ten days, and the treatment

should be accompanied by the usual local measure with special reference to the use of the Finsen or x -rays in proper dosage.

TREATMENT OF TUBERCULOSIS OF THE EYE AND EAR

Exceptionally good results have been observed in the treatment of tuberculosis of the eye and ear. Both organs, and particularly the former, are adaptable for the observation of focal reactions.

Some cases of chronic uveitis and iridocyclitis are apparently due to tubercle toxins rather than to the presence of tubercle bacilli, but in the majority of cases the eye lesions are due to the presence of the bacilli. In many cases of ocular and oral tuberculosis pulmonary lesions are likewise present, although these may be latent. The possibility of their presence, however, should always be kept in mind and especially in relation to dosage of tuberculin.

Preference should be given the bacillary tuberculins, as T. R. or B. E., in view of the excellent results reported from their use. The initial dose of either may be 0.0001 c.c., which corresponds to 0.1 c.c. of a 1 : 1000 dilution. The method of preparing the dilutions has been previously described. The injections may be given every four or five days in gradually increasing amounts, as described for the administration of tuberculin, until a dose of 0.010 or 0.1 c.c. of the tuberculin is reached. Very mild focal reactions are desirable. v. Hippel¹ starts with 0.0002 c.c. (0.2 c.c. of 1 : 1000) and gives an injection every other day in gradually increasing amounts until a dose of 0.100 (1 c.c. of 1 : 10) is reached.

TREATMENT OF TUBERCULOSIS OF THE KIDNEYS AND BLADDER

In tuberculosis of the kidney surgeons are in general accord regarding the advisability of removal of the organ as soon as possible, providing the patient has two kidneys of which the other is apparently not involved or involved so lightly that on the basis of functional tests there is good evidence for believing that it is capable of caring for elimination.

Tuberculin has been employed in the expectant plan of treatment, but with negative or indifferent results. When operation is refused or is impossible, as in bilateral infections, it may be tried; likewise after operation, although it is to be clearly borne in mind that tuberculin has no prophylactic properties as previously discussed.

In bladder tuberculosis the results of tuberculin treatment have been somewhat better; if both ureters are involved, however, great care must be exercised in dosage in order to avoid occlusion due to focal reactions.

Very mild focal reactions are desirable, but severe reactions of pain, increased frequency of micturition, and hematuria are to be carefully avoided. Dosage is also to be controlled by the state of the pulmonary lesions, which are present in many cases.

The initial dose may be 0.2 c.c. of 1 : 1000 dilution (0.0002 c.c.); subsequent doses are gradually increased at intervals of four to five days according to the plan previously outlined for the administration of tuberculin in general.

TREATMENT OF TUBERCULOUS PERITONITIS

In *tuberculous peritonitis* the results of tuberculin treatment are sometimes strikingly good. In the ascitic form drainage alone may be sufficient owing to the healing qualities of the resulting hyperemia. In the plastic form,

¹ Arch. f. Ophthal., 1904, lix, 1.

however, this procedure is not available, and fortunately tuberculin is sometimes of aid if there is no tuberculous ulcerative enteritis.

In practically all cases tuberculous peritonitis of both children and adults is secondary; bovine bacilli have been found in from 50 to 70 per cent. of cases.

The doses must be small and there is no way for observing focal reactions. Since the patients are bed-ridden, an accurate record of the temperature is possible. A dose of tuberculin should not produce an increase of temperature of more than 10 or 2° F.

For a child the initial dose may be 0.1 c.c. of 1 : 1000 dilution (0.0001 c.c.); an adult may receive 0.5 c.c. of 1 : 1000 (0.0005 c.c.) unless there is an acute primary lesion indicating a smaller dose. Skin tests are especially useful, the initial dose being the smallest amount producing a mild intracutaneous reaction.

The injections may be given every five or six days in gradually increasing amounts. When a dose has been reached under which the patient is gaining, it should be continued until progress becomes stationary, at which time it may again be increased. If hypersensitiveness supervenes during treatment the dose should be reduced.

Inasmuch as so many cases of tuberculous peritonitis are infections due to bovine bacilli, it may be a good plan to employ a bovine tuberculin and especially if human tuberculin has not proved beneficial. Raw,¹ however, believes that the tuberculins should be opposite to the infection, that is, children should receive tuberculin of human bacilli and adults, tuberculin of bovine bacilli.

SERUM TREATMENT OF TUBERCULOSIS

Various immune sera have been employed from time to time for the treatment of tuberculosis. As stated in the discussion on Immunity in Tuberculosis, it is exceedingly difficult to immunize the lower animals to the extent of producing sera containing sufficient amounts of antibodies to be even hopeful from the therapeutic standpoint. Even the immunized animal may develop the disease if too large a dose of living bacilli is given for immunizing purposes. Probably the best known sera are those of Maragliano and Marmorek:

Maragliano's serum is prepared by immunizing horses for from four to six months with a mixture of a toxin prepared by the filtration of cultures only a few days old and concentrated *in vacuo* at a temperature of 30° C., mixed with that obtained by aqueous extraction of killed virulent cultures, and concentrated by heating on a water-bath at 100° C. for three or four days. Maragliano assumes that the antiserum possesses antitoxic, bactericidal, and agglutinating properties. One cubic centimeter of this serum is injected every other day for one and a half months. The favorable action of the serum is reported on, especially by Mircoli and other Italian physicians, but in Germany and France proof of its value could not be established.

Marmorek's serum is now prepared by immunization of horses with young tubercle bacilli, whose acid-fast character is still very slight or entirely absent. When the horses have attained a high degree of immunity they receive injections of various strains of pure cultures of streptococci obtained from the sputum of tuberculous patients. The serum of these animals is, therefore, antituberculous and also antistreptococcic, and is serviceable against a mixed infection.

¹ Brit. Med. Jour., April 17, 1920, 538.

The serum is administered daily either by subcutaneous injection, in doses of from 5 to 10 c.c., or by the rectum, in doses of from 10 to 20 c.c. The latter form of administration is quite objectionable to most patients, but is the one least likely to produce serum sickness.

While this serum has been used quite extensively, the evidence at present is too conflicting to permit definite conclusions to be drawn as to its value in treatment. It would, however, seem to be worthy of further trial in cases of localized bone and joint tuberculosis and in the incipient stage of pulmonary tuberculosis. Citron recommends its use in patients who evince persistent rise of temperature, and in the very severe but not hopeless cases where tuberculin therapy cannot be undertaken. In some of these cases he has obtained very encouraging results. Citron occasionally begins with the serum treatment, and later combines tuberculin administration with it, finally omitting the serum altogether.

Porter,¹ Eversole and Lowman² have reported somewhat favorably on the use of Spengler's "I. K." serum, and Cavasse³ more recently upon Jousset's serum.

¹ Amer. Jour. Orthoped. Surg., 1911, 9, 346.

² Amer. Jour. Orthoped. Surg., 1912, 10, 234.

³ Médecine, Paris, 1920, 1, 490.

CHAPTER XLII

BLOOD TRANSFUSION

INDICATIONS AND METHODS

Historic.—While the ancients placed considerable importance upon the blood in philosophic and theologic discussions and in relation to disease, and, of course, could not have failed observing the direct consequences of hemorrhage, it is curious that no authentic records of blood transfusion are available until about 1660. There are earlier indefinite references to the subject, and it is very probable that earlier attempts were made, but without success, owing to a lack of knowledge regarding the circulation of the blood and a lack of suitable instruments and surgical skill for overcoming the difficulties arising from coagulation.

Most historians give credit to Christopher Wren, the later famous architect, for suggesting transfusion experiments following some intravenous injections which he had done in the Oxford laboratory. According to Viets,¹ an account of Wren's experiments with the assistance of Robert Boyle, the physicist, is found in No. 7 of the *Philosophical Transactions of the Royal Society* for December 4, 1665. It seems certain that as early as 1660 Boyle had given a dog an intravenous injection of opium by means of a quill, and that later Wren, Lower, and Boyle carried out blood transfusion experiments.

In February, 1665, Lower, who was an "expert anatomist" assistign Willis, transfused blood from one sheep to another, first from vein to vein and later from artery to vein, employing quills for the connections. His first demonstrations were made at Oxford, and on May 17, 1665, before the Royal Society in London, an account being recorded in the *Transactions* under date of December 17, 1666 (No. 20). During that same year Denis and Bayant, in France, performed similar experiments recorded in a letter from Denis published in No. 25, May 6, 1667, of the *Transactions*.

Samuel Pepys on November 14, 1666 recorded in his diary that "at a meeting of Gresham College, the experiment of transfusing the blood of one dog into another was made before the Society by Mr. King and Mr. Thomas Coxe upon a little mastiff and a spaniel, with very good success." On November 21st he records that "the spaniel was produced and found very well."

After Lower's public demonstration on May 17, 1665, the Society discussed the subject of priority, noting that "a Benedictine Fryer discoursed of it at M. de Monmours, ten years ago," but without further evidence to strengthen this French claim, and wisely concluding, "But whoever the Parent be, that is not so material as all that lay claim to this child should joyn together their efforts and cares to breed it up for the service and relief of human life, if it be capable of it; and this is the main thing aimed at and solicited in this Discourse."²

Lower, having blazed the trail by his animal experiments, followed this sound advice, and with the assistance of King performed the first transfusion

¹ See letter by Dr. Henry Viets in Jour. Amer. Med. Assoc., 1918, 68, 480.

² Gotch, Two Oxford Physiologists, Lower and Mayon, Oxford, 1908.

of sheep blood to a human being on November 23, 1667, at a meeting of the Royal Society at Arundel House, London, in the presence of "considerable and intelligent persons." A silver tube was used for connecting the carotid of the sheep with a vein in the subject's arm. The patient was one Arthur Coga, according to Pepys, a "poor and debauched man that the College had hired for twenty shillings to have some of the blood of a sheep let into his body, . . . their purpose to let in about 12 ounces, which they compute is what will be let in in a minute's time by the watch." The Society reports (*Transactions*, No. 30, December 9, 1667) that "the man, after this operation as well as in it, found himself very well, and hath given his own Narrative under his own hand enlarging more upon the benefit he thinks he hath received by it than we think fit to own as yet."

From this time on many attempts at blood transfusion were made, but with far more failures than successes, owing to coagulation of the blood in the cannulas and lack of sufficient skill for direct artery-to-vein anastomoses. Finally, Bischoff in 1835 introduced the method of defibrination, and from 1863 to 1884 transfusion was supposed to be a "cure-all," and the claims made for it were preposterous until Cohnheim in 1883 showed the danger of injecting defibrinated blood intravenously.

Human blood was generally employed, but sheep blood was likewise used in some instances until Landois in 1875 demonstrated that the corpuscles of these and other of the lower animals were unsuitable. Needless to state reactions were the rule and frequently fatal, but little attention was given to any of these which, at the present day, command our serious attention.

For the following twenty-five years blood transfusion commanded but little interest, the pendulum of medical opinion having swung far in the opposite direction, until Crile in 1898 revived interest in the subject by successful arteriovenous suture perfected and popularized a few years later by the work of Carrel and others. Owing to the difficulties attending vascular anastomoses it was not long before other methods employing cannulas and paraffined tubes and syringes were introduced, so that today various devices of this sort are being commonly employed for direct transfusion instead of the suture of vessel to vessel.

In the meantime the occurrence of hemolysins and agglutinins in the serum of one human being for the corpuscles of another had been discovered, the latter by Landsteiner and Shattock in 1900. The significance of these in relation to the dangers and untoward effects of transfusion were quickly appreciated by Hektoen, Crile, Ottenberg, Libman, and practically all who have written on the subject; methods were soon devised for the preliminary testing of the blood of patient and donor in order that compatible blood may be transfused, these precautions having contributed in a very important manner to the safety and comfort of the operation.

Finally, a distinct advance in blood transfusion was made by the use of sodium citrate for the prevention of coagulation almost simultaneously by D'Agote¹ of Buenos Aires, Hustin² of Brussels, and Weil³ and Lewisohn⁴ of New York. Hirudin and other anticoagulants had been previously employed, but the work of these men placed the citrate method on a firm basis and opened up blood transfusion as a therapeutic procedure within the reach of a great number of physicians lacking skill and experience for vascular anastomoses and other direct methods.

¹ An. d. Inst. mod. clín. méd., Buenos Aires, 1915, Nos. 1 and 3.

² Jour. méd. d. Bruxelles, 1914, 12, 436.

³ Jour. Amer. Med. Assoc., 1915, 64, 425.

⁴ Surg., Gynec., and Obst., 1915, 21, 37.

Since the technic of blood transfusion has become progressively more simple and our knowledge of some of the causes of untoward effects with means for their prevention advanced, the field of therapeutic application of transfusion and indications for its use have been greatly widened, and the operation no longer used only as a last resort for life-saving purposes.

TRANSFUSION FOR SIMPLE HEMORRHAGE AND SECONDARY ANEMIA

Blood Transfusion and Infusion of Other Fluids in Severe Injuries and Postoperative Hemorrhage.—Quite naturally, severe injuries accompanied by the loss of large amounts of blood were among the first conditions treated with the transfusion of blood. A large literature has accumulated on this subject, which need not be reviewed at this time, except to state that practically every writer on the subject of blood transfusion has reported upon the treatment of simple hemorrhage.

The literature records some striking examples of success, even in cases of almost complete exsanguination with apparent cessation of respiratory and cardiac activity. During the Great War blood transfusion was conducted on numerous occasions and proved a life-saving procedure with some almost miraculous recoveries, as indicated by the experiences of Bérard and Lumière,¹ Primrose,² Harrison,³ Robertson and Watson,⁴ Crabtree,⁵ and numerous others.

The question when to transfuse for acute hemorrhage depends, of course, upon individual circumstances. The drop in blood-pressure consequent to hemorrhage is sometimes life-saving, and one should hesitate raising it by transfusion until bleeding has been controlled by the tourniquet or operative procedures. As a general rule, operation is required, and especially in injuries of internal organs when no other means is available for securing the bleeding points; the accompanying shock is no contraindication to transfusion, which should be done on the table. It is well to transfuse the borderline cases with pale, cold, cyanotic, and clammy skins; weak, rapid, and thready pulse with yawning or dyspnea and a systolic pressure of 80 or less.

On the other hand, care must be exercised against "pushing the patient over" by any procedure likely to add to shock. For this reason due care must be taken to reduce the chances and degree of reaction following transfusion, with special reference to the selection of a compatible donor, the injection of whole blood by one of the direct methods being preferable to citrated blood, providing the facilities are at hand for rapid and successful work. Otherwise citrated blood may be used. The *amount* of blood to be transfused depends upon circumstances. Usually 500 to 1000 c.c. are sufficient, although a second transfusion may be required a few hours later in extreme cases. Ravdin and Glenn⁶ report the recovery of an extreme case receiving 2100 c.c. distributed over twelve hours.

If it is largely a question of restoring blood volume, as in hemorrhages of moderate degree, the infusion of warm sterile 0.9 per cent. *saline solution* may be sufficient, and has the added advantage of being less likely to produce a reaction. However, as shown by Crile⁷ and others, simple saline solution is not as efficacious as whole blood in severe hemorrhage. The latter not

¹ Presse méd., 1915, 23, 333.

² Ann. Surgery, 1918, 68, 118.

³ Lancet, 1918, 2, 455.

⁴ Ann. Surgery, 1918, 67, 1.

⁵ Boston Med. and Surg. Jour., 1919, July 17, 60.

⁶ Amer. Jour. Med. Sci., 1921, 161, 705.

⁷ Hemorrhage and Transfusion, Appleton & Company, 1909.

only maintains the nutrition of the vasomotor centers essential for the maintenance of normal blood-pressure but, being colloidal, exerts a mechanical effect in maintaining pressure because slowly diffusible, whereas simple saline solution gives an immediate rise of pressure which is not long sustained because of its non-colloidal and rapidly diffusible character.

During the Great War, when the demand for transfusion in acute hemorrhage became urgent, other substitutes for blood more efficacious and sustaining than saline solution were eagerly sought. Bayliss¹ advocated the intravenous injection of 5 to 7 per cent. solutions of *acacia*, and Hogan,² a 2.5 per cent. solution of gelatin; both of these were regarded better than plain saline solution by Hurwitz,³ Rous and Wilson,⁴ Barthélemy,⁵ and others. However, *acacia* solutions are not as harmless as was originally thought to be the case. Both Kruse,⁶ and Karsner and Hanzlik⁷ have shown that *acacia* in concentrations in which it is used intravenously may agglutinate human corpuscles not only in the test-tube, but in the living animal as well, producing emboli and anaphylactoid symptoms. Foster and Whipple⁸ have shown that *acacia* may actually reduce the coagulation time of the blood by interfering with the prompt return of fibrin to its normal value. Henderson and Haggard⁹ state that while the immediate effects from infusion of *acacia* are good, it does not improve the chances of recovery of animals subjected to the so-called "standard hemorrhage." These reports are in harmony with the unfavorable experiences of some surgeons during the war,¹⁰ and fatalities from its injection have been recorded by Olivecrona¹¹ and Lee.¹² Farrar and Ward,¹³ however, have used the *acacia* solution in some 400 odd cases, and with properly prepared solutions have had no bad results. Bayliss¹⁴ has also answered the criticisms advanced against *acacia* solutions and is convinced of the value of 6 per cent. solutions for intravenous infusion; he draws particular attention to the necessity of using a good brand of *acacia*.

As shown by Henderson, Haggard, and their colleagues, the deleterious effects of severe hemorrhage are due more to the loss of red corpuscles with consequent disturbance of the acid-alkali balance of the blood and the development of a form of asphyxia, than to the effects of fall of blood-pressure, and in their opinion transfusion of blood is the method of choice in treatment.

Transfusion for Hemorrhage Due to Ruptured Tubal Pregnancy; Placenta Prævia; Child Birth; Rupture of the Spleen and Liver, and other Injuries of Abdominal Organs.—When large blood-vessels in communication with cavities as the pelvis and abdomen, stomach and intestines and pleural sacs, or the external world, are ruptured by injury or disease, the resulting hemorrhage is usually severe enough to require blood transfusion. In ruptured tubal pregnancy the patient may become almost exsanguinated before the

¹ Brit. Med. Jour., 1918, 2, 553.

² Jour. Amer. Med. Assoc., 1915, 64, 721.

³ Jour. Amer. Med. Assoc., 1917, 68, 699.

⁴ Jour. Amer. Med. Assoc., 1918, 70, 219.

⁵ Rev. d. Clin., 1920, 39, 271.

⁶ Amer. Jour. Physiol., 1919, 49, 137.

⁷ Jour. Pharmacol. and Exper. Therap., 1920, 14, 379, 425, 449, 479.

⁸ Amer. Jour. Physiol., 1922, 58, 393.

⁹ Jour. Amer. Med. Assoc., 1922, 78, 697.

¹⁰ Central. Med. Dept. Lab. Div. Surg. Research, A.P.O., 721, October 27, 1918.

¹¹ Acta chir. Scandin., 1921, 45, 1.

¹² Jour. Amer. Med. Assoc., 1922, 79, 726.

¹³ Amer. Jour. Obstet., 1920, 1, 1; Surg., Gynec., and Obst., 1921, 32, 328.

¹⁴ Jour. Amer. Med. Assoc., 1922, 78, 1885.

pressure drops to a life-saving degree because of the capacity of the pelvis and abdomen for extravasated blood.

As a general rule there are no abnormalities in coagulation and bleeding time, except possibly in some cases of liver injury with cholemia in which coagulation may be delayed owing to deficient calcium for prothrombin inactivation. The problem is chiefly one of maintaining a sufficient number of corpuscles in circulation. When the bleeding point can be tied or constricted by operative procedures, as in ruptured tubal pregnancy, rupture or injury of abdominal organs, placenta prævia, and postpartum hemorrhage, large transfusions should be done on the table; if operation is not done, the transfusions should be smaller (250 to 500 c.c.) and repeated in order not to raise the blood-pressure too high or suddenly.

Döderlein¹ and others have stated that if the blood in the peritoneal cavity is not contaminated, as is usually the case in ruptured tubal pregnancy and rupture of the spleen, the extravasated blood may be collected into citrate solution, filtered through sterile gauze, and immediately transfused into an ovarian vein if exposed, or a vein at the elbow. If there is doubt regarding its sterility, he injects the blood subcutaneously or by rectum. As a general rule, however, surgeons prefer a donor and assistant to transfuse during the operation with the least possible delay. Titus² has advocated the routine typing of the blood of all pregnant women within six or eight weeks of expected delivery, with provisions for a compatible donor in case of necessity at a later time. This would appear to be excellent advice, and certainly a list of grouped donors should be available in maternity hospitals for call with the least possible delay in order to meet these emergencies.

Transfusion for Hemorrhage Due to Gastric and Duodenal Ulcers, Typhoid Fever, Dysentery, and Pulmonary Tuberculosis.—Ottenberg and Libman³ have shown that in the hemorrhages of gastric and duodenal ulcers, typhoid fever, and dysentery blood transfusion may not only prove a life-saving procedure, but aid in the treatment of the general state of lowered nutrition. The coagulation time is not usually increased, but they direct attention to the flabby state of the clots incapable of producing firm coagula for checking the bleeding. These investigators report that in almost all their cases of gastric and duodenal ulcers the hemorrhages stopped after transfusion, and especially those with repeated or prolonged bleeding.

Transfusion should not be done during actual bleeding in order not to raise blood-pressure, unless it is necessary to save life, in which case repeated transfusions of 250 to 300 c.c. of blood may be given. In typhoid fever cases Ottenberg and Libman have advised that upon the first appearance of blood in the stools preparations should be made for transfusion, so that if the necessity arises there may be no unusual delay.

Likewise in pulmonary tuberculosis and vascular erosions so situated that the bleeding point cannot be reached surgically, small and repeated transfusions are usually better than large transfusions in order that a low blood-pressure shall be maintained. Unfortunately, the administration of calcium chlorid does not increase the coagulability of the blood to any therapeutic degree, except possibly in obstructive jaundice, in which some effect has been noted by Schloessmann,⁴ Lee and Vincent,⁵ when administered in doses of 100 grains per day for several days.

¹ Deutsch. med. Wchn., 1920, 46, 449.

² Boston Med. and Surg. Jour., 1920, 183, 438.

³ Amer. Jour. Med. Sci., 1915, 150, 36.

⁴ Beitr. z. klin. Chir., 1912, 66, 492.

⁵ Arch. Int. Med., 1915, 16, 59.

Transfusion Preliminary to Surgical Operations and for the Treatment of Shock.—Cases of severe injuries with the loss of large amounts of blood, cases of gastric and duodenal ulcers, carcinoma of different organs, uterine fibroids with hemorrhage, etc., characterized by grave anemia and constituting bad operative risks, may be benefited and brought safely through operation by preliminary transfusion. In some instances this is accomplished by a single large transfusion or the transfusion of 600 to 700 c.c. about three days before operation, with a second transfusion on the operating-table or at the completion of the operation.

In shock due to injury and hemorrhage transfusion has proved a life-saving procedure and particularly in the experience of surgeons during the recent war; shock due to other causes may not be benefited by this measure. Harrison¹ states that transfusion in the early stages of hemorrhage shock, before the "gray-blue" stage is reached, is useful and the most efficient treatment. In the terminal or "gray-blue" stage, however, transfusion is of little use; likewise other forms of treatment. According to Cannon, shock is due to a diminution in the normal alkalinity of the blood caused by deficient oxidation, secondary to loss of red corpuscles, cardiac weakness, and low blood-pressure. In such cases the intravenous injection of simple saline solution may be followed by immediate slight improvement, but is apt to be followed by collapse within an hour later; glucose and acacia solutions have produced more lasting benefit, but in the opinion of most army surgeons transfusion and especially transfusion of whole blood by a direct method, as with Vincent's modification of the Kimpton-Brown tube, has proved the best method of treatment.

TRANSFUSION FOR HEMORRHAGIC DISEASES AND DISEASES OF THE BLOOD

The hemorrhagic diseases may be divided into those that are *primary*, including a form of bleeding of the newborn (*morbus maculosus neonatorum*), hemophilia and some of the purpuras, and those that are secondary to such blood diseases as pernicious anemia, aplastic anemia and leukemia, to hepatic disease, and various acute bacterial infections. In the primary hemorrhagic diseases the injection of blood is required primarily for promoting the coagulation of blood and to stop bleeding, and secondarily, for the correction of the resulting anemia. In the second group comprising the blood diseases, transfusion is given primarily for the temporary relief of the symptoms due to the grave anemia consequent to increased blood destruction and partial failure of regeneration, and secondarily, for stopping the hemorrhages that sometimes occur in these diseases.

Transfusion in Melena Neonatorum.—In hemorrhages of the newborn intramuscular and intravenous injections of whole blood have frequently yielded excellent results and proved a life-saving procedure. The hemorrhages may come from the nose, mouth, and gastro-intestinal tract, umbilicus, etc. Accidental hemorrhage may be due to faulty tying off of the umbilical cord.

In true hemorrhagic disease of the newborn (*morbus maculosus neonatorum*) the bleeding usually occurs in the gastro-intestinal tract with profuse and frequent vomiting and tar-like stools. The coagulation time of the blood is delayed, and, according to Whipple,² there is a deficiency in prothrombin or, at least, the elaboration of this substance is unstable.

Rabbit-, horse-, and human sera have been employed in treatment by

¹ Jour. Amer. Med. Assoc., 1918, 71, 1403.

² Arch. Int. Med., 1912, 9, 365.

subcutaneous or intramuscular injection, but, as stated in Chapter XL, much better results have followed the administration of whole blood.

Treatment should be given on the first appearance of hemorrhage. An efficient and simple procedure is to draw a 20 c.c. syringeful of blood from a vein of the father or some other healthy adult and immediately inject into the muscles of the buttocks of the child before coagulation occurs. By using a No. 18 needle for aspirating the blood a syringe may be filled in a few seconds; the injection may be given with the same or a slightly smaller needle. It is well to repeat this injection two or three hours later.

Defibrinated blood may be injected intramuscularly or subcutaneously in dose of 10 to 20 c.c. every two or three hours until bleeding has been checked; a method is described later in this chapter.

If the hemorrhages have been very copious and frequent, with signs of approaching exsanguination and collapse, transfusion of blood will be required. The injections are usually given by way of the superior longitudinal sinus. Helmholtz¹ has described a special apparatus for holding the head and transfusing whole blood by a syringe method.

Great care must be exercised against giving the injections into the substance of the brain; the method is by no means simple or devoid of danger. At least 50 to 75 c.c. of blood should be given, depending on circumstances; larger amounts may embarrass the heart muscle already weakened by anemia. Citrated blood should not be given unless means for giving whole blood are not available. I have transfused defibrinated blood filtered through gauze with complete success on several occasions. The Kimpton-Brown tube may be used for transfusing whole blood or a syringe method employing a battery of three syringes. Vincent² has described the successful transfusion of 7 cases by means of the external jugular vein and paraffin-coated tubes of proper length and size.

In none of these methods is it absolutely necessary to group the bloods of the patient and donor, inasmuch as agglutinins and hemolysins are not present in the baby's plasma. As a matter of caution, however, the writer tests the donor's serum against the baby's corpuscles before sinus transfusions (see chapter on Hemagglutinins).

Transfusion in Hemophilia.—In hemophilia blood transfusion produces prompt effects and acts as a specific agent for the treatment of hemorrhage. Unfortunately the effects are not lasting, and no means for the cure of this baffling disease have as yet been discovered. As advised by Ottenberg and Libman, *every hemophiliac ought to have at his call several persons whose blood by previous tests is known to be compatible and acceptable, and who are willing to give blood for transfusion when the necessity arises.*

According to Howell³ the hemophiliac condition, both the true hereditary type and in those instances in which the disease appears without a definite history of heredity, is due to a diminution of prothrombin with a relative excess of antithrombin; the calcium content is normal. Transfusion of normal blood supplies the deficient prothrombin for a varying period of time during which the patient is protected. Ottenberg and Libman have suggested that it may be worth while injecting a syringeful of whole blood (25 c.c.) every one to three months for prophylactic purposes.

Injections of serum have been employed in the treatment of hemophilia and apparently with success in some cases, but well-controlled studies in this disease have proved that serum, and especially old serum, is much less

¹ Amer. Jour. Dis. Child., 1915, 10, 194.

² Boston Med. and Surg. Jour., 1912, 166, 627.

³ Arch. Int. Med., 1914, 13, 76.

efficacious than blood in hemorrhagic diseases like hemophilia due to prothrombin deficiency. Lewisohn,¹ Losee,² and practically all writers on the subject of transfusion have recorded successful results. If serum is administered, it should be fresh human serum free of agglutinins for the corpuscles of the patient and given intravenously, as advised by Weil.³

Inasmuch as the hemophiliac may require several transfusions during the course of his life, the operation should be done with the least possible pain and without cutting down on the vein; the vein should be entered, if possible, by simple passage of a needle. Citrated blood has given just as good results as whole blood by a direct method, even though the chances for a reaction are greater. In slight hemorrhages one may first try the intramuscular injection of 20 to 40 c.c. of defibrinated blood, and if this fails, 100 c.c. of citrated blood should be given intravenously. As a general rule, these small amounts suffice for checking the hemorrhage, but if a large amount of blood has been lost with signs of approaching collapse, 500 c.c. should be transfused.

In necessary operations upon hemophiliacs, as the extraction of teeth, a *prophylactic* injection of blood should be given, preferably 50 to 100 c.c. of citrated blood by intravenous injection. Or 20 to 40 c.c. of blood may be injected intramuscularly with provisions made for transfusion if bleeding occurs after the operation. The intravenous injection, however, can be made less painful, although one naturally hesitates to puncture the vein of a hemophiliac. A small needle should be used (No. 20 is large enough for citrated blood), and it is my custom to pass the needle for at least $\frac{1}{2}$ inch in the subcutaneous tissues before entering the vein.

Transfusion in Purpura Hæmorrhagica and Chronic Purpura.—As shown by Duke,⁴ bleeding in acute and chronic idiopathic purpura hæmorrhagica and some other forms of purpura is due primarily to a deficiency in blood-platelets; other factors concerned in coagulation appear to be present in normal amounts. Duke has laid emphasis upon the mechanical action of the platelets and their ability to stop hemorrhage by the rôle they play in the formation of thrombi; others believe that the platelets are an important source of prothrombin and a thromboplastic substance which hasten coagulation by neutralizing the antithrombin present in normal blood. Whatever may be the mechanism of their action, and it is probable that both processes are concerned, as suggested by Hurwitz,⁵ bleeding in purpura appears to be due in most cases to a deficiency in their numbers.

For treatment fresh human serum in dose of 10 to 20 c.c. may be injected subcutaneously, but better results have followed the intramuscular injection of 20 to 40 c.c. of defibrinated human blood. In severe cases it may be necessary to transfuse 100 to 250 c.c. of citrated blood.

Transfusion in Pernicious Anemia.—No other disease has excited as much discussion relative to the value of blood transfusion as pernicious anemia. A large literature has accumulated in which the experiences of different observers have varied widely,⁶ but recent reviews of the subject have served to crystallize our knowledge of the subject and correlate experiences.

¹ Amer. Jour. Med. Sci., 1919, 157, 253.

² Amer. Jour. Med. Sci., 1919, 158, 717.

³ Trib. méd., Paris, 1907, 21.

⁴ Arch. Int. Med., 1913, 11, 100; Jour. Amér. Med. Assoc., 1915, 65, 1600.

⁵ Amer. Jour. Med. Sci., 1917, 154, 689.

⁶ For review of literature up to 1910 see article by Schultz in Crawitz, Klin. Path. d. Blutes, Leipsic, 1911, 381. For literature between 1910 and 1920 see article by Anders in Amer. Jour. Med. Sci., 1920, 158, 659.

The *cause* of pernicious anemia is still unknown; therefore our therapeutic efforts are purely empirical. The most important change appears to be the presence of a hemolytic agent capable of destroying the red blood-corpuscles more quickly than they can be produced. This factor, however, has not been identified, although it may be a living microparasite or its products. The plasma does not appear to possess increased hemolytic activity, and the location of the hemolytic agent is unknown. One naturally suspects the spleen in this relation, because it shows evidences of excessive erythrocytic activity in pernicious anemia, but the evidence is not clear or conclusive, although, as will be discussed shortly, some observers believe that removal of this organ constitutes an important therapeutic measure.

In addition to this important factor of increased blood destruction, evidence at hand indicates that there may be an acquired fault in hematogenesis on the part of the bone-marrow. In view, however, of the histologic evidences usually presented of rather strenuous efforts in this direction, it would appear to be a less important etiologic factor, and especially in the early stages of the disease.

The *purpose of treatment* is to prolong life by relieving anemia and producing frequent periods of remission. While cures have been reported, it is likely that these are explicable on the ground of faulty diagnosis or insufficient observation. Much in the way of symptomatic relief can be offered, however, except in those instances of acute virulent, rapidly progressing cases with none, or very short, remissions.

The *general plan of treatment* consists in a rigid and painstaking search for and treatment of foci of infection as emphasized by Barker and Sprunt,¹ and the administration of a full roborant diet, full doses of hydrochloric acid after meals to correct the anacidity usually present, etc. Iron may be administered by subcutaneous injection, and neoarsphenamin in small doses by intravenous injection for the effects these may have on the hemoglobin and its functions. If neoarsphenamin is administered for its general and hematinic effects, 0.1 or 0.2 gm. dissolved in 10 c.c. of sterile water and injected intravenously once a week for four or five weeks after transfusion followed by an interval, would appear to be sufficient. Of course, if syphilis is known to be present, more rigorous treatment may be required.

Unquestionably, however, the most important single therapeutic agent is blood transfusion.

The *beneficial effects and value of blood transfusion* in pernicious anemia are ascribed to a variety of possibilities: (a) The blood is probably functionally active as a transplanted tissue at least for a few weeks in the average case. (b) The transfused blood may act as a stimulant of the bone-marrow and revive for a time its flagging hematogenic properties. (c) Probably in pernicious anemia an effort is made to produce a substance in the nature of an antihemotoxin capable of neutralizing the hemotoxic agent, and blood transfusion may stimulate this process. (d) Finally, transfused blood may have a direct antagonistic effect upon the hypothetical hemolytic agent.

Whatever may be the mechanism of the beneficial effects of blood transfusion in pernicious anemia, the accumulated experiences of most observers indicate that the procedure is capable of initiating periods of remission even though the duration of these periods may not be longer than naturally occurs in the disease. But when transfusion is given early in the disease before the recuperative powers of the bone-marrow are exhausted and the degenerative changes in the spinal cord and other organs due to the anemia become appar-

¹ New York Med. Jour., June 9, 1917.

ent, these remissions may add years to life, aside from the relief from "anemic fever," increased appetite, and other general improvement that may follow. In the statistical study made by Anders remissions were induced by transfusion in 56.3 per cent. of 362 cases, and this agrees very closely with the experience of Ottenberg and Libman to the effect that transfusion initiates remissions in fully one-half of cases.

When to transfuse is usually an important question. Many patients naturally fear the operation and the possibility of disagreeable reactions. The tendency has been to delay transfusion too long; most reported cases have been transfused when the hemoglobin has reached 30 per cent. or less. This is unwarranted and uncalled for; even with the citrate method which gives a larger number of reactions than direct methods, attention to technical details and the injection of small amounts of blood at frequent intervals leaves little to be feared, and the technic is essentially simple.

Remissions are more easily induced in the early than in the late stages of the disease, and the relief from anemia will retard the degenerative effects and general symptoms directly referable to the anemia. Even when the hemoglobin falls to 20 per cent. transfusion is apt to be worth while and a means for delaying a fatal outcome, but it would appear that best results are to be expected when transfusion is initiated in the earlier stages, as soon as the diagnosis is certain and general treatment is found to be unsatisfactory, and before the hemoglobin falls below 50 per cent.

How much blood to transfuse and the frequency of transfusions are also questions of practical importance. The tendency has been to give large transfusions at irregular intervals. As previously stated, transfusion should be resorted to earlier in the disease than has been the general custom, and when the hemoglobin is not below 40 per cent. the injection of 200 to 250 c.c. of blood is sufficient, as the induction of the remission bears no relation to the amount of blood transfused. This amount of blood is less likely to produce a reaction than 500 c.c. or more, and renders the patient more willing for subsequent injections. It is likewise usually sufficient for the control of hemorrhage.

However, in cases of grave anemia, it may be necessary to transfuse 500 to 1000 c.c. of blood at least for the first time; under these conditions it is better not to use citrated blood if a direct method that does not produce too much injury to the veins of patient and donor can be employed.

The frequency of transfusion depends upon individual circumstances. After transfusion the number of erythrocytes and percentage of hemoglobin are usually increased, and an effort should be made to prevent their dropping back to pretransfusion levels as long as possible. Hemoglobin estimations, erythrocyte counts, and an inspection of the erythrocytes should be made at least once a week in periods of uncertainty and once a month under any condition. Vogel and McCurdy¹ advise that these routine examinations include a study of reticulated red blood corpuscles by means of vital staining; according to these investigators the presence of reticulated and nucleated erythrocytes are an indication of hemopoietic activity of the bone-marrow, and drop in their percentage constitutes an indication for transfusion.

The *method of transfusion* to be employed has already been briefly mentioned. The injection of defibrinated blood so popular in Germany up to recent years is not a method of choice, although if the donor is properly selected, the blood allowed to stand for an hour and filtered before injection, transfusion with defibrinated blood serves a useful purpose, and especially if only 100 to 200 c.c. are required. The intramuscular injection of 20 to

¹ Arch. Int. Med., 1913, 12, 707.

40 c.c. of defibrinated blood will usually check hemorrhage if this is the chief object of treatment.

Citrated blood has generally been employed, and when the amount of citrate is 0.2 to 0.25 per cent. the method is quite acceptable when not more than 250 to 300 c.c. of blood are required. Reactions are more likely, however, than when blood is transfused by the Kimpton-Brown, Lindemann, Unger, and such methods, but these should not be employed unless the physician possesses the necessary assistance, experience, and skill for complete success. Direct vascular anastomosis is not advisable, because the patient and donor alike may refuse subsequent transfusions by this method.

Extra care should be exercised in the selection of a *donor*. Since the plasma of the patient may possess increased hemolytic properties it is always well to match the bloods of patient and donor direct for both agglutinins and hemolysins, in addition to selecting a donor belonging to the same blood group. Every effort should be made to reduce the chances for reactions after the transfusion and to render the operation as painless and agreeable as possible. Ottenberg and Libman have advised using the same donor as long as his blood induces a remission; if the blood of a donor fails to bring about this result his blood should not be used for subsequent transfusions.

The value of *splenectomy* in the treatment of pernicious enamia is still uncertain. Ottenberg and Libman believe that this operation should be done if good remissions are no longer obtainable by transfusion alone and if the patient can be brought up to a fair standard of operative risk. For this purpose one or more transfusions may be required at intervals of three or four days before operation with another transfusion on the table.

Krumbhaar,¹ in a statistical study of 153 cases collected from literature found that the mortality was about 20 per cent. and the improvement only transient. Operative risks are increased when the hemoglobin is below 35 per cent. Lindemann,² McClure,³ Giffin,⁴ and others, however, have endorsed splenectomy as part of the treatment, and especially in young and middle-aged patients in fair general condition and in whom the spleen is moderately enlarged. There is no evidence, however, of cure, although a gain in weight and general improvement have been observed in at least 75 per cent. of cases.

In general terms, it would appear that the question of splenectomy is worthy of serious consideration and especially early in the disease when the operation risks are almost negligible. The spleen is known to show changes of increased blood destruction in pernicious anemia, and it is possible, but not proven, that splenectomy may remove at least some of the primary hemotoxic factors and processes responsible for the disease. The operation, however, should be preceded and followed by transfusions; aside from operative risks, the removal of this organ does not appear to be injurious.

Transfusion in Aplastic Anemia.—In the aplastic anemias the coagulation time of the blood is usually prolonged, and hemorrhages may require the injection of serum or blood for their control. Bleeding is apparently due to a hypofunctionating marrow as shown by the studies of Hurwitz and Drinker⁵ and the Drinkers,⁶ with a deficiency in platelets and prothrombin. According to Ravdin and Glenn, blood transfusion does not cause a remission in these cases, but rapidly repeated transfusions (twice a week) may relieve symptoms and enable the patient to conduct his affairs for a variable period,

¹ Jour. Amer. Med. Assoc., 1916, 67, 723.

⁴ Jour. Amer. Med. Assoc., 1917, 68, 429.

² Jour. Amer. Med. Assoc., 1915, 64, 613.

⁵ Jour. Exper. Med., 1915, 21, 401.

³ Jour. Amer. Med. Assoc., 1916, 67, 793.

⁶ Amer. Jour. Physiol., 1916, 41, 5.

though never a long one. Dorrance has not observed good results from transfusion in this disease.

Transfusion in Leukemia.—Hemorrhages in the leukemias are due to different factors. The coagulation time may or may not be prolonged. In the lymphatic leukemias the platelets are usually diminished, but increased in the splenomelogenous type. Antithrombin is apt to be increased in both types, while fibrinogen is absent or diminished in the lymphatic types.

Injections of blood are palliative and not curative. If hemorrhages require treatment, whole blood rather than serum should be given in order to obtain the effects of the platelets and other coagulating principles mentioned. Intramuscular injections of 20 to 40 c.c. of defibrinated blood or whole blood in a syringe before coagulation occurs may be tried; severe and recurring hemorrhages require transfusion of about 250 c.c. of blood.

Hemorrhage in Cirrhosis of the Liver; Jaundice; Acute Yellow Atrophy of the Liver.—Hemorrhages associated with acute or chronic injury of the liver are generally due to a reduction in fibrinogen. The blood coagulates, but tardily, and the clots are apt to be soft and flabby. Low fibrinogen is especially associated with cirrhosis of the liver, and Erben¹ has described the presence of a ferment in the blood in cirrhosis and certain types of myeloid leukemia, capable of producing hemorrhage by dissolving the fibrin of the clot. As previously stated, calcium is not actually diminished in obstructive jaundice, but it may be bound by the bile pigment with a functional decrease.

When surgical operations are required in the presence of jaundice and hemolytic ictero-anemia, it may be well to prepare the patient by the intramuscular injection of 20 to 40 c.c. of defibrinated human blood. Injections of serum and especially of old serum are much less efficacious. If bleeding occurs after operation or injury, or spontaneously, intramuscular injections may suffice, but if not, transfusion of 250 to 300 c.c. of blood may be required. It is possible that the beneficial results are due in part to the calcium administered in the transfused blood. Walters² has reported that the daily intravenous injection of 5 c.c. of sterile 10 per cent. solutions of calcium chlorid for three injections, combined with the administration of large quantities of carbohydrates and fluids by mouth and glucose solution by proctoclysis, enabled operations upon 34 patients with extreme jaundice without a death from hemorrhage.

Transfusion in Hemorrhages Secondary to Severe Infections in the Treatment of Septicemia, Influenzal Pneumonia, etc.—Hemorrhages may occur in the course of some of the acute infectious diseases. According to Duke³ the platelets may be diminished. In septicemia the coagulation time may be prolonged, due to an excess of antithrombin. Prothrombin and fibrinogen may be diminished. Intramuscular injections of defibrinated blood generally suffice in treatment; in severe hemorrhages blood transfusion may be required, and owing to the necessity for avoiding reactions a direct method is preferable to citrated blood if the means are available for this kind of transfusion.

Many reports have been made on the treatment of septic infections with blood transfusions, with particular reference to septic wounds and ulcerative endocarditis. Most surgeons are of the opinion that the transfusion of plain normal blood is of no value in the treatment of sepsis, although it may

¹ *Wien. klin. Wchn.*, 1902, 15, 276.

² *Jour. Amer. Med. Assoc.*, 1922, 79, 1793.

³ *Jour. Amer. Med. Assoc.*, 1910, 45, 1185.

relieve for a time the associated anemia so apt to develop in prolonged staphylococcus and streptococcus infections. Repeated small transfusions of 30 to 50 c.c. of blood may, however, aid in tiding a patient over a critical period, as indicated by the reports of Moncamy¹ and Polak.² As stated in Chapter XL transfusion of blood from donors previously immunized by few injections of vaccine may prove worth while, as indicated by the reports of Hooker,³ Wekesser,⁴ and Fry⁵ in the treatment of chronic wound infections, of Levison⁶ in the treatment of ulcerative endocarditis, and of Neuman⁷ in the treatment of typhoid fever.

In Chapter XL mention was also made of transfusions of citrated blood from convalescents in the treatment of influenzal pneumonia and other acute infections.

TRANSFUSION IN OTHER CONDITIONS

Transfusion in Acute Poisoning.—In illuminating-gas poisoning transfusion after the removal of a large amount of blood is now regarded as the best method of treatment. Phlebotomy may be done on one arm, and after the escape of 500 c.c. of blood, transfusion is started on the other arm. As much as 1000 c.c. of blood may be removed and an equal amount transfused. In severe cases a second bleeding and transfusion, or transfusion alone, may be required within twelve hours. Artificial respiration may be required.

Similar treatment may be employed in severe cases of poisoning due to other substances having their primary effect upon the blood, as benzol, nitrobenzol, etc.

Transfusion in Pregnancy; Acidosis; Diabetic Coma.—Mention has already been made of the usefulness of transfusion in the treatment of severe anemia from hemorrhage in ruptured tubal pregnancy, placenta prævia, and postpartum hemorrhage.

Bell⁸ has recently advised transfusion in the treatment of *eclampsia*, and Gettler and Lindemann,⁹ in the treatment of acute and severe *acidosis* complicating pregnancy, the donor first being prealkalinized by the administration of 20 gm. of sodium carbonate every two hours for eight doses, transfusion being done about one-half hour after the last dose.

Garnett¹⁰ has reported favorably upon the treatment of 2 cases of *per-nicious vomiting* of pregnancy by transfusion with the blood of women donors about ten days after delivery.

Ottenberg and Libman have reported upon the results of transfusion of 4 cases of *diabetes*; temporary improvement was noted in some of these, but was without effect upon the ultimate course of the disease.

Apparently transfusion has not been used in the treatment of uremia, but phlebotomy with transfusion may be worthy of trial, although only temporary effects would be expected.

Transfusion for Debilitated States.—Mention has already been made of the usefulness of transfusion for preparing cancerous individuals for operation; also for the relief of anemia and cachexia due to hemorrhages or blood destruction in other conditions requiring operative measures. Blood transfusion is thought to possess nutritive value in addition to correcting at least temporarily the faults of deficient numbers of red corpuscles and hemoglobin.

¹ Surg., Gyn., and Obstet., 1918, 27, 221.

² Amer. Jour. Obstet., 1919, 80, 291.

³ Ann. Surg., 1917, 66, 513.

⁴ Jour. Amer. Med. Assoc., 1917, 69, 2182.

⁵ Brit. Med. Jour., February 28, 1920, 290.

⁶ Jour. Lab. and Clin. Med., 1921, 6, 191.

⁷ Amer. Jour. Med. Sci., 1922, 164, 690.

⁸ Brit. Med. Jour., 1920, May 8, 625.

⁹ Jour. Amer. Med. Assoc., 1918, 68, 594.

¹⁰ Amer. Jour. Obst., 1917, 76, 303.

Fischer¹ has found transfusion useful in atrophic infants and children, and Kerley² has likewise reported increase of weight in a series of such children following transfusion with "the change from sickly, whiny infants into happy, apparently well infants. The patients were transformed from those with a digestive capacity barely able to maintain existence into those that took on the normal constructive processes of early life." Marriott³ has also recommended transfusion as a valuable measure in the treatment of "athrepsia" because of the diminished protein concentration of the plasma and the lowered blood volume found in this condition.

REACTIONS FOLLOWING BLOOD TRANSFUSION

Reactions frequently follow blood transfusion; and in severely sick individuals, especially cases of shock following injuries and hemorrhage, due account must be taken of this possibility in order to avoid fatal consequences.

The symptoms of these reactions when compatible blood is transfused, vary from a slight rise of temperature that may not be detected at all or a fever of 2° to 3° F. with malaise, nausea, and perspiration, to high fever, chills, vomiting, and urticaria. Cardiac dilatation may occur if transfusion is too rapid. The *symptoms following transfusion of incompatible blood* may have the added effects of intravascular agglutination and hemolysis. The reaction may occur during the operation or shortly afterward. Tingling body pains, precordial oppression and extreme anxiety, cyanosis, dyspnea, slow thready pulse, cold clammy skin and unconsciousness develop followed by chills, fever, and delirium. Jaundice and hemoglobinuria develop and death may occur.

The *causes* of these reactions are not well understood. Very probably more than one factor may be responsible, and the possible causes may be summarized as follows:

1. Incompatibilities of the blood of patient and donor; that is, agglutinins and hemolysins in the blood of the patient for the corpuscles of the donor or in the blood of the donor for the corpuscles of the patient. Undoubtedly gross errors in the preliminary tests or transfusion without these tests at all, may lead to accidents of this kind. In carcinoma cases, the serum of the patient may be hemolytic and result in intravascular hemolysis, despite careful preliminary tests.

2. Changes in the blood and especially of the platelets are believed important by Drinker and Brittingham.⁴ These changes resulting in an increased toxicity of the transfused blood, are believed to occur even though coagulation of the blood does not actually take place, and especially in the citrate method. As shown by the studies of Novy and De Kruif,⁵ simple defibrination of rabbit blood, or merely withdrawal of rabbit or rat blood and reinjection into the same animal or animals of the same species, may result in the acquisition of toxic properties designated by them as "anaphylatoxins" because of the kind of lesions and symptoms produced. These toxic substances may be derived from preclotting changes involving the platelets, or they may be the result of disturbed colloidal conditions. At any rate, only direct vessel-to-vessel transfusion appears to remove the possibility of their production.

3. In the citrate method Drinker and Brittingham believe that the anticoagulant promotes hemolysis of the corpuscles, even though there may

¹ Med. Record, 1916, 89, 223.

⁴ Arch. Int. Med., 1919, 23, 133.

² Amer. Dis. Children, 1917, 14, 470.

⁵ Jour. Infect. Dis., 1917, 20, 566, 589.

³ Amer. Jour. Dis. Child., 1920, 20, 461.

be no evidence of this in the test-tube. Bernhein¹ has also emphasized the dangers due to hemolysis, but according to Bayliss² hemoglobin itself is not toxic and serves as a carrier of oxygen.

4. Toxic substances may be derived from new rubber tubing in the citrate method, as shown by Bussman, unless precautions are taken to first soak it overnight in 5 per cent. sodium hydroxid followed by thorough washing.

5. In the citrate method enough of the anticoagulant alone may be injected in large transfusions to produce some untoward symptoms. The amount of citrate advised is between 0.2 and 0.3 gm. per 100 c.c. of blood. If 1000 c.c. of blood are transfused, from 2 to 3 gms. of the citrate will be given. These amounts are regarded as safe in so far as severe toxic reactions are concerned, and as much as 5 gm. may be given. For white rats I have found that 0.2 gm. per kilo of body weight represents the maximum tolerated dose, corresponding to 12 grams for an individual weighing about 130 pounds. While the intravenous injection of 2 or 3 gm. of citrate may be without danger to life, yet it is entirely likely that untoward effects may be produced.

6. The blood of one individual may act as a foreign protein when transfused to another and initiate the changes and reaction described in Chapter XXXIX.

7. Finally, some reactions may be anaphylactoid due to some effect of foreign protein, as suggested by Bayliss and also by Bowcock.³ It is possible that with intravascular hemolysis the hemoglobin may not be toxic, but the corpuscular fragments may produce embolic or colloidal anaphylactoid lesions and symptoms. The possibility of acquired sensitization and true anaphylactic reactions is present, if the blood proteins of the donor undergo sufficient change to act as a foreign protein.

This possibility is suggested by clinical observations, showing that after repeated transfusions and especially in pernicious anemia, the patient acquires a tendency for reactions, and particularly if the same donor is employed. Apparently agglutinins and hemolysins capable of demonstration in the test-tube do not develop, but an increased tendency toward reactions may become apparent, as reported by Sydenstricker, Mason, and Rivers.⁴ Bowcock has also described the occurrence of these reactions in cases of pernicious anemia, and states that transfusion may become self-limited in this disease because of the inadequacy of methods for selecting suitable donors. Meleny, Stearns, Fortune, and Ferry⁵ have likewise observed this acquired tendency, and believe that the blood of one donor may be more likely to produce reactions than others.

The effects of transfusion upon the blood of the patient are variable, and probably due to the mechanical results in relation to the quantity of blood transfused as well as to reactive effects upon the patient's blood-making organs. As shown by the studies of Huck,⁶ transfusion is usually followed by an immediate increase of red blood-corpuscles, but not in proportion to the amount of blood injected. In some cases this increase lasts only for a few hours, in others for twenty-four hours followed by a drop to the pre-transfusion level, and in others the increase reaches its maximum in twenty-four to forty-eight hours and remains higher than before transfusion for a variable time. The hemoglobin is generally increased, reaching the maximum in twenty-four hours, but these changes are not necessarily in propor-

¹Lancet-Clinic, 1915, 113, 253.

²Brit. Jour. Exper. Path., 1920, 1, 1.

³Bull. Johns Hopkins Hosp., 1921, 32, 83.

⁴Jour. Amer. Med. Assoc., 1917, 68, 1677.

⁵Amer. Jour. Med. Sci., 1917, 154, 733.

⁶Bull. Johns Hopkins Hosp., 1919, 30, 63.

tion to the red corpuscle changes. The leukocytes are almost always increased with an increase of the polymorphonuclear neutrophils; in some cases, however, there are no changes or even a decrease. As stated by Huck, it may be said that transfusion raises the count, but the effects are essentially biologic, involving the redistribution of blood in the body, and the exact nature of the changes are not understood.

CHOICE OF METHODS FOR BLOOD TRANSFUSION

Until about ten years ago blood transfusion was an exceedingly difficult procedure involving artery-to-vein anastomoses and requiring refined surgical technic and considerable skill and experience; even under these conditions the operation frequently failed, and the ordeal was one which most patients refused to undergo a second time.

With the introduction of syringe-cannula and paraffin-coated tube methods the technic of transfusion of whole blood was greatly simplified, but the introduction of anticoagulants and especially sodium citrate, brought the operation within the reach of many more physicians.

All methods have certain advantages and disadvantages which may be summarized as follows:

1. **Direct Artery-to-vein or Vein-to-vein Anastomosis.**—The chief and only, but important, advantage is the transfusion of blood practically unchanged from its condition in the donor. Reactions are almost eliminated, or at least reduced to less than 10 per cent.

The disadvantages are the chances of failure due to clotting, leakage, etc. Also the fact that cutting is required, with pain, fright, and nervousness on the part of both patient and donor. The method is not at all adapted for cases requiring repeated transfusion.

Furthermore, it is impossible to measure the amount of blood transfused, although Libman and Ottenberg¹ have described a method for approximating the amount. Dorrance² has recorded one instance where a back flow from patient to donor must have occurred, with consequent infection of the donor. This method has, therefore, little to recommend it, and has been largely discarded, as, likewise, modifications of the method whereby the vessels are united by buttons or tubes.

2. **Syringe, syringe-cannula, paraffin tube, and special methods** of a similar kind for the transfusion of unmodified blood have the advantage of producing fewer reactions and injecting nothing but blood free of anticoagulant. When it is especially advisable to avoid reactions, as in cases of severe hemorrhage with shock and cases of primary and secondary anemia in an extreme state of exhaustion, when a reaction may prove to be the "final push," these methods are especially valuable, as recently stated by Bernheim.³ Lindemann,⁴ Losee,⁵ Unger,⁶ Lundblad,⁷ and others prefer these methods, and the Vincent modification of the Kimpton-Brown tube was used quite extensively in the American, French, and British armies during the World War.

As stated by Unger, when the indications are to replace diseased with normal blood for the purpose of deriving the benefits of the latter as a transplanted tissue, whole unmodified blood is to be preferred to citrated blood.

¹ Jour. Amer. Med. Assoc., 1914, 62, 764.

² Penn. Med. Jour., 1922, 25, 245 (discussion).

³ Jour. Amer. Med. Assoc., 1921, 77, 275.

⁴ Jour. Amer. Med. Assoc., 1916, 66, 624.

⁵ Amer. Jour. Med. Sci., 1919, 158, 711.

⁶ Jour. Amer. Med. Assoc., 1921, 77, 2107.

⁷ Penn. Med. Jour., 1922, 25, 245.

There can be no doubt, however, that all of these methods are more complicated than the citrate method. They require experience, skill, and trained assistance, and especially the Ziemssen syringe method and its modification by Lindemann, and the Unger method. The Kimpton-Brown tube can be worked by one operator, and with a properly prepared tube is an excellent method.

3. The **citrate method** has the great advantage of simplicity. Any physician practised in intravenous therapy can make it work satisfactorily. No special apparatus is required; any apparatus suitable for intravenous infusion may be employed, although special apparatus is advisable for preventing accidental contamination of the blood during its collection and administration.

Since the introduction of this method in 1915, and especially by Lewisohn, it has easily become the most popular, although its value is reduced by certain disadvantages.

In addition to the ease with which blood is collected and administered, this method has the additional advantage of producing little or no pain, as it is unnecessary to cut down on the vein of either patient or donor, except, possibly, in children and obese adults. Furthermore, the transfusion can be done with the minimum of fuss and excitement, and the donor need not be brought in contact with the patient. Blood may be collected at one place and infused some hours later, or even next day at another place. It is especially useful for the transfusion of nervous and apprehensive patients, as the injection of blood can be given at the bedside.

The method is well suited for the treatment of hemorrhage unless the patient is shocked, when the effects of a reaction are to be borne in mind.

Bernheim states that the citrate method produces reactions in 20 to 40 per cent. of cases, as compared with 5 per cent. or less reactions when unmodified blood is transfused. Taking a rise of temperature of 2.5° F. as constituting a reaction, Drinker and Brittingham observed 53.3 per cent. reactions, with chills in 40 per cent. Pemberton, however, observed reactions in but 21 per cent. of 1036 transfusions. Meleny and his associates observed reactions in 70 per cent. of cases transfused with citrated blood, as compared with 26.4 per cent. of reactions when unmodified blood was transfused by the Lindemann syringe-cannula method. There can be no doubt, therefore, that citrated blood is more likely to produce reactions than unmodified blood; in addition, Unger states that citrate renders the corpuscles more fragile, diminishes complement in the donor's serum, and reduces the phagocytic activities of leukocytes. However, according to Mellon, Hastings and Casey,¹ Unger's statements concerning the destructive influence of sodium citrate upon complement, phagocytic activity, and opsonins have not been confirmed by their experiments, and an explanation for the reactions attending the use of citrate must be sought along other lines. According to Lewisohn² a mixture of sodium citrate with blood in the proportion of 0.25 per cent. does not affect the erythrocytes or leukocytes and does not increase the percentage of post transfusion chills.

Other anticoagulants have been employed, especially hirudin by Satterlee and Hooker,³ and others, but this substance is apt to be more toxic, due, according to Marshall,⁴ to products of putrefaction. Potassium oxalate, so commonly employed for the prevention of coagulation of blood for chemical

¹ Proc. Soc. Exper. Biol. and Med., 1922, 19, 344.

² Jour. Amer. Med. Assoc., 1923, 80, 247.

³ Jour. Amer. Med. Assoc., 1914, 62, 1781; *ibid.*, 1916, 66, 618.

⁴ Jour. Pharmacol. and Exper. Therap., 1915, 7, 157.

analysis, is too toxic, and should never be used. In my experiments it was found that the maximum tolerated dose of this substance for white rats by intravenous injection was about 0.025 gm. per kilo as compared to 0.200 gm. of sodium citrate. It is, therefore, six to ten times more toxic than sodium citrate, although slightly more anticoagulating. It is generally necessary to use 0.2 per cent. potassium oxalate for the prevention of coagulation and 0.2 to 0.3 per cent. sodium citrate, but potassium oxalate is so much more toxic and dangerous than sodium citrate that mention is made of it only as a matter of precaution against its use.

4. The **method of defibrination** is not now generally employed for large transfusions, although it is very useful for the intramuscular injection of small amounts of blood for the control of hemorrhage.

Defibrination is usually conducted by whipping the blood with sterile glass rods or collecting about 100 c.c. in each of a series of flasks and shaking with sterile glass beads. It is always advisable to filter the blood before intravenous injection.

The method has the advantage of simplicity, although it is cumbersome and apt to be messy. Reactions may follow, and many investigators, including O'Connor,¹ Brodie,² Sollmann,³ and Stewart and Harvey,⁴ have shown that pressor and other toxic substances develop after defibrination and clotting. Hürter⁵ states that the therapeutic effects of whole blood are distinctly better than those following the use of defibrinated blood.

SELECTION OF DONORS

Donors should be preferably large, strong, muscular men with prominent veins at the elbow. Nervous, apprehensive, and obese individuals should not be used, and especially in syringe-cannula methods for the transfusion of unmodified blood, as they may be unable to keep quiet at critical times.

If a professional donor is employed, due care must be exercised against employing one with anemia due to too frequent transfusions. If doubt exists regarding the state of the blood, the erythrocytes should be counted and hemoglobin estimated. In transfusion for hemophilia, if a relative is employed, extra care must be exercised in the choice of the donor.

In case of professional donors it is well or have them sign a legal form relieving patient and physician of further responsibility, and stating the stipend received.

The donor should be free of tuberculosis, syphilis, or other communicable disease; there should be no evidences of cardiac disease. If time permits the Wassermann reaction should be conducted. Sydenstriker, Mason, and Rivers have recorded the probable transmission to patients of syphilis and malaria by blood transfusion.

During the transfusion the donor should be watched for sweating, yawning, and pallor, as collapse may follow. In the citrate method very little injury is done the vein, usually nothing more than a needle puncture, and the donor may resume his usual activities the following day. If the vein is exposed by incision, a week or more may be required for healing.

For infants, the mother or father, or practically any healthy adult may be used as a donor.

¹ Arch. f. exper. Path. u. Pharmacol., 1912, 67, 195.

² Jour. Physiol., 1900, 26, 48.

³ Amer. Jour. Physiol., 1905, 13, 291.

⁴ Jour. Exper. Med., 1912, 16, 103.

⁵ Med. Klinik, 1911, No. 12.

PRELIMINARY COMPATIBILITY BLOOD TESTS

As previously stated, the blood of a proposed donor may contain agglutinins and hemolysins for the corpuscles of the patient, and if his blood were transfused, dangerous reactions would very probably follow the operation. Or these substances may not be in the blood of the donor, but in the blood of the patient for the corpuscles of the donor, in which case the donor would be unsatisfactory for the transfusion.

In former years severe reactions were due to the transfusion of such incompatible bloods; even today some physicians minimize the danger and go ahead with transfusion regardless of the presence or absence of these substances. In grave emergencies this is justifiable when time for the matching tests cannot be spared because the good that may be done by prompt transfusion under the circumstances offsets the possible harm. But the tests require very little time and should always be done if at all possible. Surgeons of wide experience, like Bernheim and Dorrance, who at first ignored these tests and who gave many transfusions without untoward results, now regard them as absolutely essential after having had some unfortunate experiences.

Many observers have stated that agglutination tests alone are sufficient, and that if these substances are absent in the blood of the donor for the corpuscles of the patient and in the blood of the patient for the corpuscles of the donor, the bloods may be regarded as compatible, because hemolysins are not present if agglutinins are absent. In a general way this is true and undoubtedly the agglutination tests are more important than the hemolysin tests and may be relied upon alone; but, as shown by Ottenberg and Kaliski,¹ hemolysins may develop in disease. Losee states that in the treatment of hemorrhage due to injury, the patient being otherwise healthy, agglutination tests alone are sufficient, but that in disease both agglutination and hemolysin tests should be conducted. As a matter of fact, both tests can be done together in about the same time required for the agglutination tests alone, and when the patient is suffering with pernicious anemia or carcinoma, the hemolysin tests should always be included. According to Levine and Segall² prolonged etherization may cause a temporary change in the blood grouping.

The subject of hemagglutinins is discussed in Chapter XVI, and methods described for conducting these preliminary agglutinin and hemolysin tests. These tests should be conducted with great care and both Dykes³ and Ottenberg⁴ have recently emphasized the importance of using only active Group II and III serums, since error may be due to the use of old and weak serums; Dyke recommends using only such serums as are able to cause definite agglutination when diluted 1 : 10.

As is now well known, the blood of human beings is divisible into four groups on the basis of agglutination tests, and it is easily possible to determine to which group any individual belongs. After childhood the group does not change; if an adult is found to belong to Group I his corpuscles will always remain in that group throughout life.

Unfortunately, two methods of grouping are now in vogue, namely, that of Jansky and that of Moss. Each recognizes four groups; Jansky's Group I corresponds to Moss's Group IV and Jansky's Group IV to Moss's Group I. Groups II and III correspond in both. *It is essential that the method is*

¹ Jour. Amer. Med. Assoc., 1913, 61, 2138.

² Surg., Gyn., and Obstet., 1922, 35, 313.

³ Brit. Jour. Exper. Path., 1922, 3, 146.

⁴ Jour. Amer. Med. Assoc., 1922, 79, 2137.

known and recorded when an individual's blood is grouped. For example, a patient may be found belonging to Group I by the Moss classification; the donor must be Group I by the same classification, as a Group I donor by the Jansky method would be incompatible. If the grouping of both patient and donor is done in the same laboratory, confusion is not apt to occur; but if the patient is grouped and a donor selected on the basis of a grouping done elsewhere, great care must be exercised against the possibility of error.

It is generally regarded as sufficient to select a donor belonging to the same group as the patient. But if time permits *it is always well to directly match the bloods of patient and donor before transfusion* by mixing the serum of the patient with the corpuscles of the donor and the serum of the donor with the corpuscles of the patient. The technic is described in Chapter XVI. Thalheimer¹ and others have emphasized the necessity of conducting these direct tests, and especially when the patient has been transfused on a former occasion. Mention has already been made of observations by McClure and Dunn² and others, indicating that in pernicious anemia repeated transfusions sometimes result in more and more difficulty in finding a perfectly compatible donor. While there is no evidence to show that injections of human corpuscles into human beings lead to the formation of agglutinins and hemolysins, yet this has been found to occur by Robertson and Rous³ in the lower animals, and changes may be brought about in human beings, although their nature is not at all understood. *Furthermore, subgroups to the main groups of hemagglutinins are now known to occur, and these may be detected and minor incompatibilities prevented by the direct tests in addition to the group tests.* Guthrie and Huck⁴ have recently studied this important subject of subgroups with particular care and have presented evidence that the popular belief in the existence of four and only four iso-agglutinin groups is incorrect. By direct tests and by absorption experiments they believe that they have demonstrated the existence of three instead of two iso-agglutinins and three instead of two iso-agglutinogens, with these three iso-agglutinins and three iso-agglutinogens there are possible at least 27 biologic combinations; 8 of which they believe to have observed, instead of but the four groups or combinations recognized at present in the Jansky and Moss classifications. For this reason some of the post-transfusion reactions occurring after the injection of bloods of the same groups may be due to the presence of undetected agglutinins; more than ordinary care is required in the blood-matching tests, and Guthrie and Huck have suggested that the grouping should be determined on a basis of the agglutinin-agglutininogen content, since only bloods with the same agglutinin-agglutininogen content belong to the same group.

Individuals belonging to Group I (Jansky) or Group IV (Moss) are commonly regarded as universal donors suitable in case of emergency for the transfusion of anyone. Under these conditions reliance is based upon the fact that the high dilution to which the incompatible plasma is brought may nullify its toxic effect, but during the World War the Interallied Surgical Conference⁵ stated that fatal accidents have followed, although the chances of eliciting these were slight, and that such donors may be used in emergencies.

¹ Jour. Amer. Med. Assoc., 1921, 76, 1345.

² Bull. Johns Hopkins Hosp., 1917, 28, 99.

³ Jour. Exper. Med., 1922, 35, 141.

⁴ Bull. Johns Hopkins Hosp., 1923, 34, 125.

⁵ Presse méd., 1918, 26, 193.

PREPARATION OF THE PATIENT AND DONOR

No special preparation is required. Losee recommends that the patient be given 900 c.c. of milk in divided amounts twenty-four hours before transfusion in order that the heart may become accustomed to increased volume of fluid. It is well that the bowels of both patient and donor be empty and likewise the bladder of each, in view of the nervousness of both during the operation. A nervous patient may require $\frac{1}{8}$ gram of morphin about one-half hour before operation.

The donor should not have eaten within two hours of operation; I believe that reactions are sometimes caused by the transfusion of chylous plasma.

It is not necessary to prepare the arms beforehand, and every effort should be made to prevent undue confusion and fuss; it is easily possible to protect the patient and donor against nervous strain and fear.

POSTTRANSFUSION TREATMENT

During the transfusion both patient and donor should be watched closely, especially the patient during the injection of the first 100 c.c. of blood, and the donor after 500 c.c. of blood have been removed.

If the patient shows signs of distress, as cyanosis, dyspnea, slow pulse, clammy skin, etc., as previously described, the transfusion should be interrupted and 1 c.c. of 1 : 1000 adrenalin and 1/100 grain of atropin sulphate administered subcutaneously. If these effects pass off, the transfusion may be carefully and slowly resumed. If they do not pass away or recur, the transfusion should be stopped, as the donor is very probably incompatible.

After transfusion the patient usually develops fever and perspires; this does not require special treatment. If a severer reaction occurs, the treatment is symptomatic. Urticaria may be relieved by local applications or the subcutaneous injection of adrenalin, as described in Chapter XXXII for the treatment of serum sickness.

The donor usually feels little or no effects if 500 c.c. or less blood are removed. He should be carefully watched, however, for yawning, pallor, nausea, cold clammy skin, and a weak pulse. These effects are usually psychic, and a kind, sympathetic attitude on the part of the operator with words of encouragement, are usually all that is required for their prevention. When larger amounts of blood are removed, the donor may feel faint; and if so, removal of blood should be stopped at least for a few minutes and aromatic spirits of ammonia or whisky offered. It is well for the patient to rest for a few hours after removal of blood, although the majority refuse to do so.

FATE OF THE TRANSFUSED BLOOD

As previously stated, the effects of transfusion on the patient are due to the mechanical presence of so much new blood, to stimulation of the patient's blood-making organs, and a redistribution of blood through the body. As shown by Ashby,¹ the length of time the transfused blood remains in circulation varies greatly and elimination varies greatly and takes place in cyclic crises rather than as a continuous process.

Rous and Robertson² have shown that the transfused corpuscles are gradually broken up and the fragments filtered out in the blood-stream. Doubtless some of the cells are phagocytosed by fixed and wandering cells of the patient; this undoubtedly occurs when incompatible bloods are transfused, as shown by Ottenberg and others.

¹ Jour. Exper. Med., 1921, 34, 127.

² Jour. Exper. Med., 1917, 25, 651.

Just how long the transfused corpuscles continue to function in their new host is not possible to state. In pernicious anemia and other hemolytic diseases their duration is probably much shorter than in the blood of a healthy host treated for hemorrhage. Even before they are removed from the circulation the cells may lose in hemoglobin content and be functionally inactive. But if there is no immediate break down of the donated corpuscles, they may be demonstrated in the blood for more than thirty days, and according to the results obtained by Ashby employing an agglutination method, for as long as three months even in pernicious anemia. This leads Ashby to conclude that the primary beneficial effects of transfusion are not due to a stimulating effect upon the bone-marrow, but more probably to the functioning of the transfused red blood-corpuscles. These results have recently been confirmed by Wearn, Warren, and Ames,¹ who found that red blood-corpuscles from donors in Group IV transfused into patients in Group II with pernicious anemia and anemia secondary to nephritis, remained in the circulation longer than has been generally believed to be the case. The last of the transfused red blood-cells disappeared from the circulation in from fifty-nine to one hundred and thirteen days, with an average of eighty-three days. No difference was noted in a series of observations in the duration of the stay of the transfused red blood-corpuscles in the circulation between patients with primary anemia and secondary anemia (due to nephritis). In a single observation red blood-corpuscles from a patient with pernicious anemia transfused into another patient with pernicious anemia, behaved as did the corpuscles from normal donors.

TRANSFUSION WITH PRESERVED RED BLOOD-CORPUSCLES

Rous and Turner² have shown that the red blood-corpuscles of rabbits may be kept in mixtures (of 3 parts of blood in 2 parts of 3.8 per cent. sodium citrate in water with 5 parts of 5.4 per cent. saccharose in water) for fourteen days; the mixtures were kept in a refrigerator, and before transfusion the supernatant fluid was pipeted off and the corpuscles suspended in fresh sterile Locke's solution. When used to replace normal blood the transfused corpuscles remained in circulation and functioned as well as fresh corpuscles. Cells kept for longer periods, though intact and apparently unchanged when transfused, soon left the circulation, although without producing harm.

Robertson³ used this method for the preservation of human corpuscles and transfused 20 individuals suffering with hemorrhage, shock, and sepsis, chiefly hemorrhage and shock. Considering the type of cases given these transfusions, the results were good and equal in Robertson's opinion to transfusions of fresh blood. Four weeks represented the limit to which human blood could be kept in the above solution in a refrigerator. The advantages were the simplicity of technic, having blood on hand at the minute it was needed, and that the transfusion could be given without assistance.

METHODS FOR BLOOD TRANSFUSION

A large number of methods for blood transfusion have been described. Some are quite complicated and others simple, doubtless all have proved satisfactory in the hands of their inventors.

(A) *Suture and Cannula Methods for Direct Transfusion.*—The Carrell suture, Crile and Elsberg cannula methods are difficult surgical operations. Pope⁴ has described a simple cannula method composed of two glass tips

¹ Arch. Int. Med., 1922, 29, 527.

² Brit. Med. Jour., 1918, 1, 691.

³ Jour. Exper. Med., 1916, 23, 239.

⁴ Jour. Amer. Med. Assoc., 1913, 60, 1284.

connected with a short piece of rubber tubing, the whole being boiled in equal parts of petrolatum and paraffin before use for sterility and coating. A similar apparatus has been more recently described by Fullerton, Dreyer, and Bazett¹ for direct transfusion. It is necessary in these methods to cut down and expose the vessels of both donor and patient in order to insert and tie in the cannulas.

(B) *Syringe and Syringe Cannula Methods for Direct Transfusion.*—The oldest syringe method is that of Ziemssen; the Lindemann² syringe-cannula method is practically a refinement of this method and is described later on. Abelman³, Brown,⁴ and Lintz⁵ have described special syringe methods; and Unger⁶ has likewise devised an apparatus for drawing blood from the donor and at once injecting it into the patient by the operation of a system of stop-cocks.

Cooley and Vaughan⁷ and Howard⁸ have described very simple syringe methods for transfusing children with whole or citrated blood, the technic being described later on.

(C) *Paraffined Tube Methods for Direct Transfusion.*—The method of Kimpton and Brown⁹ is best known and yields excellent results; it will be described later under Technic. Percy¹⁰ and Kreuscher¹¹ have also described similar methods.

(D) *Citrate Methods.*—Lewisohn¹² has described a very simple method, but it is open to the objection of the blood being subject to contamination. The apparatus devised independently by Hoffmann and Habein¹³ and Robertson¹⁴ are closed and remove this objection. My own apparatus, described later, is almost identical with these. Hartman¹⁵ has also described a special jar for transfusion of citrated blood.

TECHNIC OF BLOOD TRANSFUSION

Since in the practice of blood transfusion some cases may be satisfactorily transfused with citrated blood, while others should receive unmodified blood, two methods for each with which the writer has had best results, are described.

Lewisohn's Citrate Method.—*Obtaining the Blood from the Donor.*—A tourniquet is applied to the donor's arm, and one of the larger veins in the elbow region (usually the median cephalic vein) is punctured. A cannula of large diameter (13 gage) is used in order that the blood may flow out rapidly through the needle. For guiding the flow of blood a Luer adapter with short rubber tubing is inserted in the needle hub. The blood is collected in a graduated glass jar which contains at its bottom the 2.5 per cent. citrate solution. If 450 c.c. of blood is to be given, add 50 c.c. of this solution, thus affecting a 0.25 per cent. mixture. Smaller and larger amounts of blood are treated proportionately. This technic is illustrated in Fig. 194.

Infusion of the Blood Into the Recipient.—The recipient is usually so anemic that the vein must be exposed by a small incision in about 80 per cent. of the cases. A cannula 15 gage is inserted, and the latter is attached to the gravity tube which contains from 20 to 30 c.c. of physiologic sodium

¹ Lancet, 1917, 1, 715.

² Amer. Jour. Dis. Child., 1913, 6, 28.

³ Jour. Amer. Med. Assoc., 1916, 67, 142.

⁴ Jour. Exper. Med., 1918, 28, 623.

⁵ Jour. Amer. Med. Assoc., 1913, 61, 171; Boston Med. and Surg. Jour., 1918, 178, 351.

⁶ Surg., Gyn., and Obstet., 1915, 65, 269.

⁷ Jour. Amer. Med. Assoc., 1918, 70, 223.

⁸ New York State Jour. Med., 1919, 19, 431.

⁹ Jour. Amer. Med. Assoc., 1921, 76, 358.

¹⁰ Brit. Med. Jour., 1918, 1, 477.

¹¹ Jour. Amer. Med. Assoc., 1916, 66, 506.

¹² Jour. Amer. Med. Assoc., 1915, 65, 1027.

¹³ Jour. Amer. Med. Assoc., 1913, 60, 435.

¹⁴ Jour. Amer. Med. Assoc., 1915, 65, 1365.

¹⁵ Jour. Amer. Med. Assoc., 1918, 71, 1658.

chlorid solution. The blood is then poured into this apparatus and allowed to run into the punctured vein by gravitation (exactly like an ordinary saline solution). In newborn infants a Kaliski cannula, 18 gage represents the proper size (Fig. 195).

The apparatus required for this method may be purchased as a complete outfit, but can be improvised with perfectly satisfactory results. A disadvantage is that there are chances for air contamination of the blood and especially during its collection. For that reason the following method was devised:

Author's Citrate Method.—As previously stated, this method is fashioned after that of Robertson and Hoffmann and Habein.

The apparatus is shown in Fig. 196 and serves both for the collection of blood and its injection under aseptic conditions. One operator can do the work, but it is better done with an assistant.



FIG. 194.—TAKING BLOOD FROM THE DONOR. (LEWISOHN'S METHOD.)

The apparatus is readily assembled and consists of a 1000 c.c. filter flask graduated into divisions of 100 c.c. each and fitted with a two-holed rubber stopper, glass tubes, rubber tubing, gage No. 18 needles, and a Potain aspirating syringe. The assemble and dimensions of all parts are shown in Fig. 196 and need not be further described. Tubes B and C with attached needles are enclosed in test-tubes, and the whole sterilized by autoclaving. The 50 c.c. graduated cylinder (H) carries a sterile 5 per cent. solution of sodium citrate in water. This is added to the blood in the proportion of 5 c.c. per 100 c.c. of blood, giving a concentration of 0.25 per cent. citrate. An advantage of the apparatus is that citrate is added under aseptic conditions, as required, in order to avoid an excess. Even with large transfusions of 900 c.c. the total amount of citrate would be 45 c.c. of the 5 per cent. solution or 2.25 gm., which is well within the limits of safety.

The technic is as follows:

1. A tourniquet is applied around the arm of the donor, and he is requested to vigorously open and close the hand for the purpose of rendering

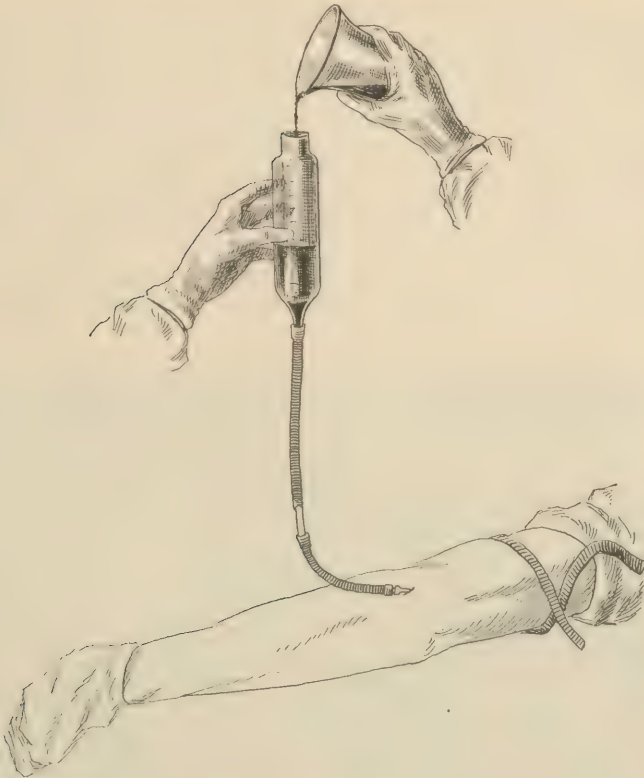


FIG. 195.—INFUSION OF CITRATED BLOOD INTO THE RECIPIENT. (LEWISOHN'S METHOD.)

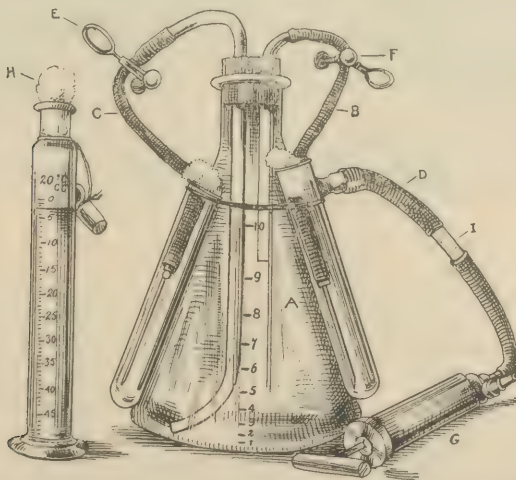


FIG. 196.—APPARATUS EMPLOYED BY THE AUTHOR FOR THE COLLECTION, CITRATING, AND INFUSION OF BLOOD.

A, A 1000 c.c. filter flask; B, tubing and No. 18 needle for collection of blood (enclosed in a test-tube for sterilization); D, tubing with filter (I) and syringe (G) for exhausting air during collection and pumping air for the infusion; C, tubing with needle (No. 18) for citrating blood (H) during collection and later for infusion of the mixture into the patient; E and F are stop-cocks.

the superficial veins full and tense. The skin is cleansed and one or two coats of tincture of iodine applied.

2. Tube C with its needle are removed from the test-tube and the end placed in the cylinder of 5 per cent. citrate solution. Stop-cock E is left open, but F is closed. The syringe G is applied to tube D and suction made, drawing 10 c.c. of the citrate solution into the flask, which is sufficient for the first 200 c.c. of blood.

Stop-cock E is now closed, but during the collection of blood it is opened, as required, and measured amounts of the citrate solution permitted to enter the flask.

3. Tube B with its needle is removed from the test-tube and the needle inserted into a prominent vein of the donor. Stop-cock F is open, but E is



FIG. 197.—COLLECTION AND CITRATION OF BLOOD.

closed. Suction is made with the pump to keep up a negative pressure in the flask, but continuous pumping is not required, leaving the hands free to gently shake the flask from time to time to mix the blood and citrate solution. Blood flows in a steady stream (Fig. 197). As the 200 c.c. mark is reached, stop-cock E is opened and 5 c.c. of citrate solution permitted to enter at the same time, which is sufficient for 100 c.c. more of blood. In this manner citrate is gradually added *beforehand* as required.

4. The arm of the patient is prepared in the same manner. Stop-cock F is closed throughout. The syringe G is reversed to pump air into the flask, which is filtered through I. Stop-cock E is opened and the pump worked until tube C and its needle are filled with blood. Cock E is then closed until the needle has been entered into the vein, when it is opened, the tourni-

quet released, and the blood gently pumped in (Fig. 198). Care must be exercised against too vigorous pumping, and it is advisable to wire the stopper into the flask to avoid loosening during the infusion. *As the flask is about emptied, due care must be exercised against admission of air by closing-cock E and removing the needle from the vein.*

Some of the advantages of this apparatus are as follows: (a) The negative pressure in the flask during the collection of blood prevents coagulation in the needle and tubing until the required amount of blood is obtained. This also permits the use of a smaller needle than ordinarily employed. (b) The blood is collected and citrate added under closed and aseptic conditions. (c) Citrate is added as required in order to avoid an excess, as sometimes happens if enough is placed in a flask for 500 c.c. of blood, but only 200 to 300 c.c. of blood actually obtained. (d) It provides a uniform

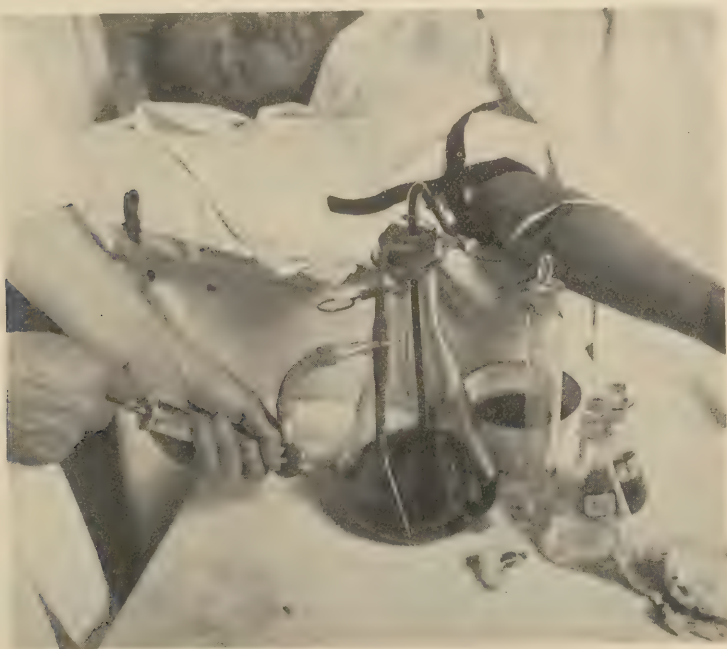


FIG. 198.—INFUSION OF THE CITRATED BLOOD.

steady flow of blood. (e) With a little practice the whole operation may be done by one person.

Kimpton-Brown Method.—This is a satisfactory method for the transfusion of *unmodified* blood and especially for adults. It depends upon the principle of using a coating of paraffin to prevent contact of blood with glass and thereby preventing coagulation for a sufficient time to permit transfusion. I have applied this method as follows:

1. The tube is shown in Fig. 199 and usually comes in two sizes, 250 c.c. and 150 c.c., although larger or smaller ones may be prepared. I have found it convenient to slightly enlarge the tip and attach about $\frac{3}{4}$ -inch of rubber tubing, carrying an adapter for the Record syringe needles.

2. The tube is wrapped in a towel and autoclaved. The paraffin (melting-point 54° C.) or better, Vincent's mixture of 1 part stearin, 2 parts

of paraffin, and 2 parts vaselin, is sterilized and melted by boiling in a water-bath or autoclave.

The tube is carefully and moderately heated over two alcohol lamps. The stopper is removed and the tube half-filled with the hot fluid paraffin mixture, which is rolled around inside the tube and over the surface of the cork, and a little permitted to run out the cannula's end (needle detached). The tube is now emptied through the side tube, leaving a *thin* but complete coat of paraffin mixture inside. The tube is now cooled by sponging with alcohol, and is ready for use. Two or three such tubes should be in readiness, and may be prepared beforehand and kept sterile wrapped up in sterile towels.

3. It is worth while to attach a piece of rubber tubing to the side arm and carrying a glass window filled with cotton for a filter. This tubing is sterilized by autoclaving. During the collection of blood the operator can apply suction if necessary to aid filling by negative pressure. During the

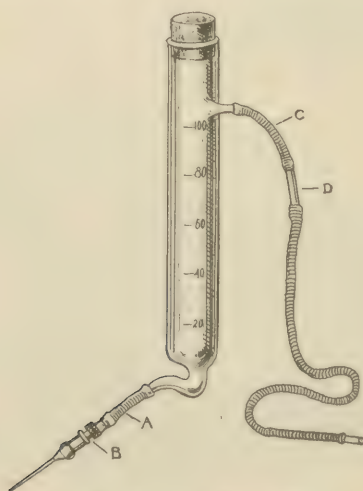


FIG. 199.—KIMPTON-BROWN TUBE.

This shows a 100 c.c. size with tubing (C) and mouth-piece attached with air filter (D) for suction during collection of blood if necessary. A short piece of tubing (A) has been attached in such manner that the glass tip of the tube almost touches the adapter B fitting a 1-inch No. 16 Record needle.

injection of blood a bulb syringe may be attached and filtered air pumped into the tube to facilitate emptying.

4. Donor and patient should be lying on adjoining tables or beds, and their arms prepared.

A tourniquet is applied to the arm of the donor and a vein at the elbow rendered full and tense by opening and closing the hand. A Record needle (gauge No. 16, 1 inch in length) is inserted into a vein, and the adapter end of the tube attached. The tube fills with blood by venous pressure, care being taken not to have the tourniquet too tight or too loose. A blood-pressure cuff is best for keeping the blood-pressure at about 60 to 65. Filling may be facilitated as necessary by suction on the rubber tubing attached to the side tube. If a needle is not employed, the skin over the vein should be infiltrated with sterile novocain, an incision made, the vein exposed, and the usual ligatures applied. The vein is opened and the cannula end of the tube inserted for about $\frac{1}{2}$ to 1 inch, and held upright until it fills. I prefer

the needle arrangement as obviating the necessity for cutting in most instances and facilitating the filling of two or three tubes with least loss of blood.

5. The tube is now detached from the needle and a second tube immediately attached and filled. The first tube may be handed to an assistant, who proceeds with the injection, or it is held in such a way as to prevent loss of blood until the second and third tubes have been filled, when the tourniquet is released and the needle withdrawn.

6. The tourniquet is now applied to the patient, and a vein at the elbow rendered full and tense if this is possible. If the anemia does not permit this, it may be necessary to cut down on the vein under local anesthesia, but this should be determined beforehand and done if necessary, so that there may be no delay in injecting the blood after the tube or tubes have been filled. I have used Lindermann's cannula with excellent results for insertion in the vein of the patient, although a plain needle of the above-mentioned dimensions is satisfactory for the donor.

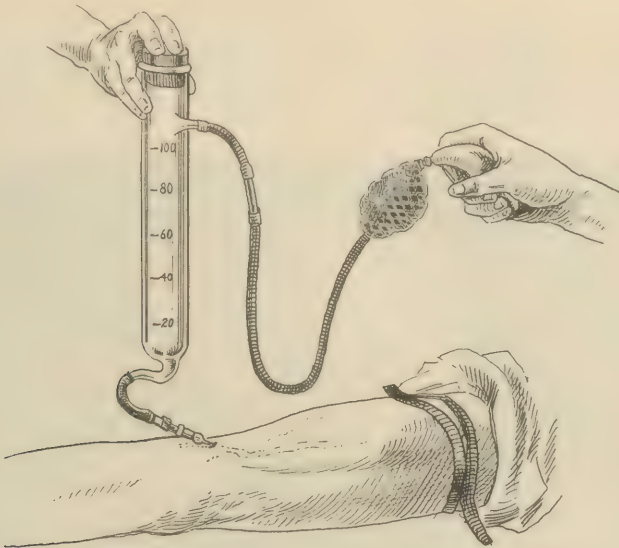


FIG. 200.—INJECTION OF BLOOD WITH THE KIMPTON-BROWN TUBE.

If a vein is full and sufficiently tense a No. 16 needle (1 inch length) or Lindemann cannula is inserted direct, the tourniquet released, a tube attached, and the blood infused, the flow being aided by gentle pumping with a caudery bulb or some other suitable bulb (Fig. 200). After one is nearly emptied, the succeeding ones are attached, the needle being left in place until the infusion is completed.

The Lindemann Syringe Cannula Method.—This method is for the transfusion of unmodified blood. Its special feature is the needle-cannula (Fig. 201) by means of which cannulas are inserted into the veins of donor and patient without exposing the veins. In addition to two of these cannulas, three or four 25 c.c. Record syringes are required, and all sterilized by boiling for a few minutes just before use.

1. One operator draws the blood, a second injects, and a third cleanses the syringes. Patient and donor should be on adjoining tables, with an

instrument table between upon which the outstretched arms are placed; the third assistant stands between with a basin of warm sterile saline solution, washing the syringes and handing them forward and backward between the operators (Fig. 202).

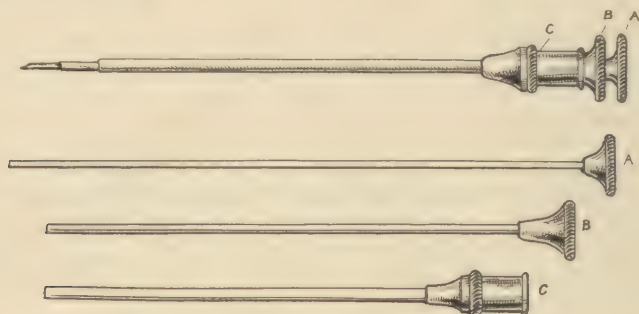


FIG. 201.—THE LINDEMANN CANNULA.

2. The arm of the donor is prepared and a tourniquet applied. A cannula is gently inserted, the trocar (A) being sharp, until cannulas B and C are in the vein for at least a length of $\frac{3}{4}$ to 1 inch. Trocar A and cannula B are



FIG. 202.—BLOOD TRANSFUSION WITH LINDEMANN CANNULAS AND SYRINGES.

removed, leaving cannula C in place. A 25 c.c. Record syringe filled with warm sterile saline solution is attached, and the assistant maintains a very slow injection to prevent escape of blood and coagulation.

3. The second cannula is inserted into the vein of the patient, the tourni-

quet released, and a syringe containing warm saline attached to prevent loss of blood and coagulation.

4. The first operator now fills his syringe with blood, detaches, and immediately connects a second syringe. The second operator detaches his syringe carrying saline, attaches the syringe filled with blood, and injects. The third assistant washes out the syringes and hands them to the first operator, etc., until the required amount of blood has been transfused.

I have sometimes used a sterile 5 per cent. solution of sodium citrate in water for washing the syringes instead of plain saline solution; this tends to prevent coagulation in the syringes, and the very small amounts of citrate injected do not materially modify the method.

In adults and most children over two years of age the median basilic vein may be employed.

Transfusion of Infants.—The Lindemann method may be employed, the cannula being inserted into the external jugular or internal saphenous veins. Not more than 50 to 100 c.c. of blood are usually required. According to Halbertsma¹ an injection of 15 c.c. of blood per kilogram of body weight produces a rise of 1,000,000 corpuscles and that this affords a means for calculating beforehand the amount of blood to be transfused.

A citrate syringe method may be employed as follows:

1. Three or four 25 c.c. Record syringes are sterilized and 2 c.c. of a sterile 5 per cent. solution of sodium citrate in water placed in each.

2. The arm of the donor is prepared and a tourniquet applied. A needle (gage No. 18 is usually large enough) is inserted into a vein and the syringe almost filled with blood. A second syringe is quickly attached and filled, followed by a third and fourth if required. The tourniquet is now released and the needle removed. This method is similar to that described by Howard.²

3. Coagulation is prevented if the citrate and blood in each syringe are mixed.

4. Or the blood may be citrated by placing 5 c.c. of 5 per cent. citrate solution in a sterile bottle or small Erlenmeyer flask and emptying the syringes as they are filled. This amount of citrate is sufficient for 100 c.c. of blood. If less blood is required, 1 c.c. of the citrate solution is sufficient for each 20 c.c. of blood. As a general rule, there should be three syringes, so that an assistant may empty a syringe, wash it out once or twice by drawing up and expelling warm citrate solution, and place it beside the operator, who fills and detaches without undue haste. Or the method described by Zingher³ may be employed.

5. The injections are now given through the superior longitudinal sinus or external jugular vein, a smaller needle being employed. The technic is described in Chapter II and illustrated in Figs. 21 and 23.

6. Recently Siperstein and Sansby⁴ have advocated *intraperitoneal* injections of citrated blood in the transfusion of infants. In experiments employing rabbits they have found that blood is rapidly absorbed from the peritoneal cavity and that intraperitoneal transfusion of freshly citrated blood acts as a true transfusion and not merely as the absorption of a nutrient material. Siperstein⁵ has subsequently reported upon the treatment of 5 cases, favorable results being obtained in 3. This route of

¹ Amer. Jour. Dis. Child., 1922, 24, 269.

² Jour. Amer. Med. Assoc., 1915, 65, 1365.

³ Medical Record, March, 1915, 13.

⁴ Amer. Jour. Dis. Child., 1923, 25, 107.

⁵ Ibid., 1923, 25, 208.

transfusion is simple, practical, and efficient according to these investigators, and while it is not the route of choice, it is suggested as a therapeutic procedure of merit when the anterior fontanel is closed and surgical exposure of a vein is difficult.

Intramuscular Injections of Blood.—Several methods may be employed; all are quite simple and efficient.

1. According as 20 or 40 c.c. of blood are required, one or two 25 c.c. Record syringes are sterilized, 2 c.c. of 5 per cent. solution of citrate placed in each, the syringe or syringes filled with blood from the donor, the contents mixed, and the intramuscular injections given as described in Chapter XXXVII and illustrated in Fig. 181.

2. Or the required amount of citrate solution (2 c.c. of a 5 per cent. solution for each 20 to 25 c.c. of blood) is placed in a sterile bottle, blood aspirated from a vein of the donor, and expelled into the bottle. As a general rule only one syringe is required, as it may be easily filled twice without washing between fillings, a finger closing the end of the needle to prevent bleeding while the syringe is being emptied. After the blood is secured the tourniquet is released, the syringe washed out once or twice with saline solution, and the injection given.

3. Or one, two, or more syringefuls of blood may be collected and expelled into a sterile bottle or flask containing glass beads, shaken for a few minutes for defibrination, and the blood injected intramuscularly.

4. Or, if the patient is placed close at hand and only 20 to 25 c.c. of blood are required, a syringe may be collected, using a gage No. 16 or 18 needle to facilitate quick filling, the tourniquet released, needle and syringe withdrawn, and the blood injected at once (but not too rapidly) before it has had time to coagulate in the syringe.

PART V

EXPERIMENTAL INFECTION AND IMMUNITY

INTRODUCTORY

Methods.—The exercises and experiments herein outlined are for the purpose of teaching the principles of infection and immunity by actual laboratory work, whereby the student performs the experiments and is taught to observe the results. At the same time a knowledge of the technic of immunologic methods is obtained. The instructor in charge of such an experimental course may choose certain experiments in outlining a course according to the allotment of time and purpose of the instruction.

In all instances I have outlined the experiments according to average conditions. It is readily understood that differences in the virulence of a certain culture or the weight and physical condition of an experimental animal will require that the doses advised be changed to meet the conditions.

As a general rule, a course should be concentrated, with exercises at least three times a week in order that the student may follow his work closely and have an opportunity for making adequate and accurate observations.

An attempt is made to bring out the important points of an experiment by a few pertinent questions. It should be impressed upon the student that the mind should be held open for observation and that unexpected and untoward results may be obtained which, however, are always of interest and always instructive when the experiment is conducted in a careful, methodical, and conscientious manner. Frequent general discussions should be held for a general review of the subject and correlation of facts and observations. In my experience students are eager for the work and seldom fail to suggest additional work in the nature of original research.

The Student.—1. The student should work protected by an apron or gown with short sleeves; he should be careful of the hands and avoid abrasions and cuts, and carefully wash and disinfect the hands after the work of each day has been completed.

2. The working table should be set in order after each day's work; pipets and soiled glassware should be properly disposed of, instruments thoroughly cleansed, and the table wiped off with 1 per cent. formalin solution. It should be impressed upon the student that good and accurate work is seldom done amid disorderly and dirty surroundings.

3. The student must never sacrifice accuracy for speed; painstaking and accurate work is always to be desired; speed is gained only with practice.

Records.—Each worker should record in writing in a suitable notebook his observations of the various tests and experiments. Not infrequently unexpected results are obtained, and it is important to understand and explain these as correctly as possible. Note-books should be subject to frequent inspection; it is not necessary to write out a description of the technic, but the results of the experiment and answers to the questions should be set forth clearly and with sufficient detail.

Animal Experiments and Autopsies.—In all experiments calling for operative procedure an anesthetic is to be used in order that unnecessary pain be avoided. Ordinarily, ether is to be employed, or in rabbits the

rectal injection of 0.5 to 1 gram of chloral hydrate dissolved in 5 to 10 c.c. of water. Autopsies are to be carefully conducted, and the lesions described in writing. After autopsy the table is to be scrubbed and cleansed with a solution of formalin, and the carcass disposed of by incineration or placing in a solution of formalin until removed and otherwise disposed of.

ACTIVE IMMUNIZATION OF ANIMALS

EXPERIMENT 1.—PRODUCTION OF AGGLUTININS, BACTERIOLYSINS, AND OPSONINS:

1. Secure a culture of *Bacillus typhosus*, an old laboratory culture of cholera bacilli, and a culture of *Staphylococcus aureus*.
2. Proceed to immunize two rabbits with each culture by intravenous injections according to the technic described in the text.
3. One week after the last injection the animals are to be bled and the serums secured after the methods described in the text.
 - (a) Define the meaning of antigen.
 - (b) What are antibodies?
 - (c) What is meant by active immunization?
 - (d) What precautions should be observed in handling these antigens?
 - (e) What precautions are to be observed in giving intravenous injections?
 - (f) What antibodies do you suspect are present in these immune serums?

EXPERIMENT 2.—PRODUCTION OF PRECIPITINS:

1. Immunize two rabbits with sterile horse-serum by intravenous injections after the methods described in the text.
2. Immunize two rabbits with sterile human serum.
3. Bleed the animals from the carotid artery one week after the last injection and preserve the serums in a sterile condition.

EXPERIMENT 3.—PRODUCTION OF HEMOLYSINS:

1. Immunize a rabbit with washed sheep corpuscles by intravenous injections after methods described in the text.
2. Immunize a second rabbit with sheep cells.
3. Immunize a third rabbit with washed human cells.
4. Immunize a fourth rabbit with washed human cells by intraperitoneal injections.
5. Bleed the animals in four days to a week after the last injection and separate the serums.
6. Prepare a portion of human amboceptor dried on paper as described in the text.
7. Preserve the balance of the serum and the other serums with equal parts of neutral glycerin.

EXPERIMENT 4.—PRODUCTION OF CYTOTOXIN:

1. Immunize two rabbits with dog kidney after the method described in the text.
2. After the immunization has been completed, preserve the serum with 0.2 per cent. phenol in sterile ampules.

While these various immune serums are being prepared the student is engaged with the work on infection, or if the subject of immunity is taken up at once, immune serums should be furnished by the instructor.

EXPERIMENTAL INFECTION

EXPERIMENT 5.—EXPERIMENTAL PNEUMONIA:

1. Grow a virulent culture of pneumococcus in glucose serum broth for forty-eight to seventy-two hours at 37° C. until a good rich growth is secured. Prepare and stain smears by Gram's method.

2. Pass a large catheter which has its tip cut off into the trachea of a dog until it has passed into one of the primary bronchi. By means of a syringe inject quickly 15 c.c. of culture; remove the catheter and mouth-gag. Take the rectal temperature and leukocyte count previous to inoculating.

3. Inject a rabbit with 5 c.c. of culture by tracheotomy under anesthesia.

4. Inject a second rabbit with 5 c.c. of culture intravenously.

5. Observe all animals for forty-eight hours, taking the rectal temperature night and morning. Make leukocyte counts every four hours during the day. Make physical examination of the chest.

6. Autopsy the animals under aseptic precautions and with complete anesthesia.

7. Culture the heart's blood of each in serum bouillon.

8. Culture the pulmonary lesions in serum bouillon.

9. Prepare smears of the heart's blood and lesions and stain with methylene-blue and Gram's stain.

10. Remove consolidated portions of lung and place in 5 per cent. formalin. After twenty-four hours cut sections which are passed through by the paraffin method and stained with hematoxylin and eosin, methylene-blue, and Gram's stain.

(a) Did any of the animals show evidences of infection?

(b) Are there evidences of pneumonia? How do these lesions compare with those of human pneumonia?

(c) How do you explain their production?

(d) Does the rabbit receiving the dose of pneumococci intravenously show evidences of pneumonia? If not, why not?

(e) Were the temperature changes similar to those observed in human lobar pneumonia?

(f) Did leukocytosis occur, and if so, why?

(g) Are there any evidences of pleuritis, and if so, how do you explain its production?

(h) Are the pneumococci seen in the smears of the lesions and blood encapsulated? If so, what is the significance of these capsules? Compare these cocci with those shown in the smear of the culture before injection? Are the capsules lost in the artificial culture-media?

(i) Discuss the question of the possible modes of infection in human lobar pneumonia.

EXPERIMENT 6.—RELATION OF NUMBERS OF MICROPARASITES TO INFECTION:

1. Secure a white rat infected with *T. equiperdum* and showing large numbers of the micro-parasites in tail blood examined microscopically.

2. With a red corpuscle pipet, a diluting fluid (2 c.c. glacial acetic acid, 2 c.c. formalin, 2 c.c. carbolfuchsin, and 96 c.c. of water), and a counting chamber, determine the number of parasites per cubic centimeter of blood.

3. Place 10 c.c. of 4 per cent. sodium citrate in water in a 25 c.c. graduated cylinder. Etherize the rat and bleed from the vessels of the neck, collecting the blood by means of a small funnel into the citrate. Mix well and calculate amount of blood secured and approximate number of trypanosomes per cubic centimeter.

4. Prepare a dilution with warm saline solution in such manner that 1 c.c. contains 100,000,000 trypanosomes; a second solution to contain 1,000,000 per cubic centimeter; a third to contain 100,000 per cubic centimeter; a fourth to contain 10,000 per cubic centimeter; a fifth to contain 1000 per cubic centimeter; and a sixth to contain 100 per cubic centimeter. Inject 1 c.c. of each intraperitoneally into 6 rats respectively of approximately equal weights.

5. Examine 1 drop of blood from the tail of each animal eighteen and twenty-four hours later and daily thereafter.

6. When the animals succumb, remove the brain, liver, and spleen of each; fix in formalin, prepare and stain sections.

(a) Does the time of appearance of trypanosomes in the peripheral blood bear a relation to numerical infection? Does the duration of life?

(b) Discuss the probable causes of death in these animals.

BACTERIAL, PLANT, AND ANIMAL TOXINS

EXPERIMENT 7.—DIPHTHERIA TOXIN AND TOXOID:

1. Secure diphtheria toxin or prepare by inoculating tubes of neutral or slightly alkaline bouillon with a virulent culture of diphtheria bacilli (Park-Williams Bacillus No. 8 being especially desirable).

2. Grow at 35° C. for five days and filter the culture through a Berkefeld filter.

3. Inject a 250- to 300-gram guinea-pig in the median abdominal line with 0.5 c.c. of the filtrate (toxin).

4. Heat 1 c.c. of toxin at 60° C. for an hour and inject 0.5 c.c. into a second guinea-pig subcutaneously.

5. Observe the animals for at least four days, especially for the development of a characteristic edema about the site of injection.

6. After death perform a careful autopsy, paying particular attention to the bloody edema at the site of injection and marked hyperemia of the suprarenal glands. Make cultures on Löffler's blood-serum medium of edematous area, peritoneum, and heart blood.

(a) To what has death been due?

(b) Has diphtheria toxin a selective affinity for any particular tissue?

(c) Has heat any effect upon diphtheria toxin?

(d) Is there a period of incubation before symptoms develop, and why?

EXPERIMENT 8.—METHOD OF TESTING THE VIRULENCE AND TOXICITY OF DIPHTHERIA BACILLI:

1. Make a culture of a patient harboring the bacilli on a tube of Löffler's serum medium. Inoculate at 35° C. for from eighteen to twenty-four hours; prepare a smear and stain with Löffler's methylene-blue. If diphtheria bacilli are present, they must be isolated in pure culture. *Never attempt a guinea-pig test with an impure culture.*

2. Isolate by the "streak" method, on plates of blood-serum.

3. Inoculate a tube of 1 per cent. glucose bouillon, which is neutral or slightly alkaline, with several different colonies.

4. Incubate at 35° C. for three days, keeping the tube in a slanted position in order to give the culture as much oxygen as possible. If a good growth does not appear in twenty-four hours, transplant to another tube of bouillon until the bacilli have been "educated" to grow on the medium.

5. Examine for purity. Select a 250- to 300-gram guinea-pig and inject 2 c.c. of the unfiltered culture in the median abdominal line. Animals over the weight specified are more resistant and less reliable for test. The unfiltered culture is used, since toxin is but one element of the disease-producing power of diphtheria bacilli, and toxin production in bouillon may not be a true index of the toxin production in mucous membranes.

6. Carefully observe the animal for at least four days. Even slight toxemia, especially if accompanied by edema at the site of injection, should be regarded as a positive result.

7. After death perform a careful autopsy. Make cultures of the edematous area, peritoneum, and heart blood. Observe whether acute hyperemia of the suprarenal glands is present.

8. Not infrequently animals showing mild or even an absence of the symptoms of toxemia develop paralysis of the hindquarters two or three weeks later. According to Ehrlich, this paralysis is due to the action of "toxon," a toxic substance secreted by the bacillus or, as believed by others, a modified form of toxin.

9. To prove that diphtheria was the cause of the toxemia or death, mix 2 c.c. of the culture in a test-tube with 1 c.c. of diphtheria antitoxin (500 units). After standing aside for an hour at room temperature, inject the mixture subcutaneously in the median abdominal line of a 250- or 300-gram guinea-pig.

(a) Is the diphtheria bacillus aggressive?

(b) What evidence have you that the lesions and death are due to a toxin?

(c) Why does the use of diphtheria antitoxin make the test conclusive? Would tetanus antitoxin be capable of neutralizing diphtheria toxin?

EXPERIMENT 9.—TETANUS TOXIN; TETANOSPASMIN:

Tetanus toxin is composed of two distinct poisons of different properties. One, *tetanospasmin*, has a great affinity for the central nervous

system, and is largely responsible for the symptoms of tetanus infection (neurotoxic); the second, *tetanolysin*, is thermolabile and is hematoxic. Tetanus toxin is very labile, and when in solution soon becomes attenuated. For these experiments it is necessary to use either fresh toxin or that which has been recently precipitated and dried.

1. Secure some dried tetanus toxin and dissolve in sterile salt solution. The toxin may be secured from an antitoxin laboratory and preserved indefinitely in the refrigerator. Since the strength varies with different products, the lethal dose for a 300-gram guinea-pig should be ascertained from the laboratory furnishing the toxin.

2. Secure 2 grams of fresh normal guinea-pig brain and liver. Crush in separate sterile mortars.

3. Add a lethal dose of fresh tetanus toxin and 5 c.c. sterile salt solution to each. Mix thoroughly and place in the incubator for an hour. Remove and carefully transfer to sterile centrifuge tubes. Centrifuge thoroughly.

4. Inject two 300-gram guinea-pigs subcutaneously in the median abdominal line with the supernatant fluids.

5. Inject a third guinea-pig with a similar dose of toxin and 5 c.c. salt solution (control).

6. Mark pigs carefully and observe for several days.

(a) What are the symptoms of tetanus?

(b) To what constituents of tetanus toxin are the chief symptoms due?

(c) Does the pig which received the toxin-brain mixture show symptoms?

How do you explain the result?

(d) Does the pig which received the toxin-liver mixture show symptoms?

How do you account for the result?

(e) Is the tetanus bacillus characterized by its toxicity or aggressiveness?

(f) Is there a period of incubation before symptoms develop, and why?

(g) Autopsy the animal. Are there any definite lesions of the tissues and internal organs?

EXPERIMENT 10.—ACTION OF TETANUS ANTITOXIN; SPECIFICITY OF ANTITOXINS:

1. In a test-tube or syringe place 50 M. L. D. of tetanus toxin and 2 c.c. of saline solution.

2. In a second test-tube or syringe place 50 M. L. D. of tetanus toxin and 2 c.c. of tetanus antitoxin.

3. In a third test-tube or syringe place 50 M. L. D. of tetanus toxin and 2 c.c. of diphtheria antitoxin.

4. After one hour inject the mixtures subcutaneously into three young guinea-pigs respectively.

5. Observe the animals for four or five days.

(a) Discuss the mechanism of neutralization of tetanus toxin by antitoxin.

(b) Discuss the specificity of antitoxins.

(c) Define the units of tetanus and diphtheria antitoxins.

EXPERIMENT 11.—TETANUS TOXIN; TETANOLYSIN:

1. Secure some fresh tetanus toxin.

2. Prepare a 1 per cent. emulsion of washed sheep corpuscles (washed three times in an excess of normal salt solution).

3. Into a series of six test-tubes place 1 c.c. of the corpuscle emulsion and increasing doses of toxin: 0.1, 0.2, 0.4, 0.8, 1, and 2 c.c. Add sufficient normal salt solution to bring the total volume to 3 c.c.

4. Place 1 c.c. of the corpuscle suspension in 2 c.c. salt solution as a control to make sure that the salt solution is isotonic.

5. Heat a portion of toxin at 60° C. for an hour in a water-bath and set up a similar set of tubes.

6. Incubate all tubes for two hours at 37° C.

7. Read results at the end of this time and again after the tubes have settled in the refrigerator overnight.

- (a) Has hemolysis occurred in any of the tubes?
- (b) What is meant by hemolysis?
- (c) What constituent of tetanus toxin is responsible for hemolyzing these cells?
- (d) Does this experiment show the selective affinity of a toxin for certain cells?
- (e) How is tetanus toxin produced?
- (f) Does heat destroy the hemotoxic agent?

EXPERIMENT 12.—ANTITETANOLYSIN:

1. Having determined the hemolytic unit of the tetanus toxin, place 2 units in each of four small test-tubes.

2. Add tetanus antitoxin to each of the first three in the following amounts: 0.1, 0.5, and 1 c.c. To the fourth tube add 1 c.c. of saline solution.

3. In a fifth tube place 2 c.c. of saline (to be the corpuscle control).

4. Place tubes in a water-bath for one-half hour.

5. To each of the five tubes add 1 c.c. of a 1 per cent. suspension of washed sheep corpuscles; mix and reincubate for one hour.

- (a) In which tube has hemolysis occurred, and why?
- (b) Is tetanus antitoxin capable of neutralizing tetanolysin?

EXPERIMENT 13.—BOTULISM TOXIN:

1. Prepare toxin by cultivating the *Bacillus botulinus* in an alkaline bouillon made in the form of an infusion from ham with the addition of 1 per cent. of glucose, 1 per cent. of peptone, and 1 per cent. of sodium chlorid. Strict anaërobic cultures should be grown for four weeks and filtered through a Berkefeld filter. The toxin may be preserved in brown, sealed vials, and kept on ice, or in a dried form in vacuum.

2. Dilute 0.2 c.c. of the toxin with 3 or 4 c.c. salt solution and administer, per os, to a cat by means of a small catheter passed into the stomach.

3. Inject 0.1 c.c. of the toxin subcutaneously in the abdominal wall of a rabbit.

4. Observe animals closely for several hours following administration of the toxin, for some products are so highly toxic that symptoms may appear within a few hours.

- (a) What are the symptoms of botulism infection?
- (b) Do these symptoms show any selective action of botulism toxin for certain tissues?
- (c) Autopsy the animals. Are there any gross tissue changes?

EXPERIMENT 14.—DYSENTERY EXOTOXIN AND ENDOTOXIN:

1. To plain meat infusion broth add one-third volume of a 10 per cent. solution of egg-albumen. Adjust to $\text{pH} = 7.6$ to 7.8 and autoclave for forty-five minutes at a pressure of 15 pounds.

2. Inoculate heavily with Shiga dysentery bacillus and incubate at 37°C . for five days. Filter through a sterile Berkefeld N candle. The filtrate is to be tested for exotoxin (Olitsky and Kligler).

3. Inject two young rabbits intravenously with 0.5 and 1 c.c. of the toxin. Heat toxin at 70°C . for one hour and inject a third rabbit with 1 c.c.

4. Cultivate the bacillus on Blake bottles of agar for twenty-four hours. Wash off each growth with 15 c.c. of saline solution and incubate the suspension at 37°C . for two days. Filter through a Berkefeld N candle (endotoxin).

5. Inject two young rabbits intravenously with 2 and 5 c.c. of the toxin. Heat toxin at 70°C . for one hour and inject a third rabbit with 5 c.c.

(a) Observe the animals for evidences of muscular weakness, prostration, paralysis, loss of weight, and diarrhea.

(b) Carefully examine rabbits postmortem and particularly for pathologic changes in the peritoneum, intestines, brain, and spinal cord.

(c) Do the two toxins act alike? Which produces lesions and symptoms referable to the nervous system? Which produces lesions and symptoms referable to the intestinal tract?

(d) Do the toxins vary in resistance to heat?

EXPERIMENT 15.—BACTEREMIA; EMBOLIC ABSCESES:

Inject 5 c.c. of a twenty-four-hour bouillon culture of a virulent strain of *Staphylococcus aureus* in the ear vein of a rabbit. Take the temperature before and every twelve hours after injection; also leukocyte counts. Autopsy the animal seventy-two hours later under aseptic precautions.

(a) What lesions are found?

(b) Where are these chiefly situated, and why?

(c) Culture the heart's blood on tubes of agar, also several of the lesions.

(d) After twenty-four hours' incubation examine your cultures.

(e) What was death due to?

(f) Define bacteremia.

(g) Prepare and stain sections of the kidney, including a lesion. What are the histologic changes? How do you explain the production of these tissue changes?

(h) What is meant by focal infection and selective localization of bacteria? What physical factors may influence the localization of bacteria in the tissues?

EXPERIMENT 16.—STAPHYLOLYSIN:

1. Inoculate a flask of neutral bouillon with a virulent culture of *Staphylococcus aureus* and grow for several days at 37° C.

2. Prepare a 1 per cent. suspension of washed rabbit erythrocytes in normal salt solution.

3. Into a series of six test-tubes place 1 c.c. of the corpuscle suspension and increasing amounts of *staphylococcus* culture: 0.1, 0.2, 0.4, 0.8, 1, and 2 c.c. Add normal salt solution to bring the total volume to 3 c.c.

4. Prepare a control by placing 1 c.c. of corpuscle suspension in 2 c.c. salt solution; also a second control by mixing 1 c.c. of sterile bouillon and 1 c.c. of corpuscle suspension.

5. Incubate tubes for two hours at 37° C. and read results.

6. Repeat, using human, rabbit, and guinea-pig erythrocytes.

7. This test will be referred to again in the technic for determining the antilysin in blood-serum.

(a) Has hemolysis occurred in any of the tubes?

(b) To what constituents of the culture are these results due?

(c) Does this experiment show the selective action of a bacterial toxin?

(d) Do the results have any bearing upon the anemia and jaundice of severe *staphylococcus* infections?

(e) What other bacteria contain or produce hemolysins?

EXPERIMENT 17.—STREPTOTOXIN; STREPTOLYSINS:

1. Cultivate a strain of virulent hemolytic streptococci in tubes of slightly alkaline serum or ascites bouillon for forty-eight hours at 37° C.; also on blood-agar plates.

2. Inoculate a guinea-pig intraperitoneally with 1 or 2 c.c. of the unfiltered culture.

3. Test for the presence of streptolysins, using sheep corpuscles and increasing amounts of unfiltered culture as in Experiment 16. Repeat, using human, rabbit, and guinea-pig corpuscles.

4. Autopsy aseptically eighteen or twenty-four hours later.

(a) What are the gross features of the exudate?

(b) Prepare smears of the exudate and stain with methylene-blue. Are there many cells present? How do you explain the cellular content?

(c) Are streptococci present? Are these inclosed by any of the cells? How do you explain the condition?

(d) Is the exudate bloody? If so, why?

(e) Examine your blood-agar plates. Are there any peculiar changes around the colonies? Do any of the test-tubes show hemolysis? To what are these changes due? Does this have any connection with the bloody character of the exudate?

(f) Why are streptococcus infections so virulent and spreading in character?

(g) Is the streptococcus an aggressive micro-organism?

(h) Do streptococci contain an endotoxin?

(i) How are streptococci classified?

EXPERIMENT 18.—PHYTOTOXINS:

1. Secure 0.01 gm. ricin or abrin and dissolve in 10 c.c. normal salt solution or distilled water.

2. Inject 1 c.c. intravenously into a rabbit. Place the animal in a metabolic cage and collect urine, or catheterize every twelve hours or at death.

3. Examine the urine for hemoglobin and erythrocytes.

4. Prepare a 1 per cent. suspension of washed rabbit and guinea-pig corpuscles.

5. Into a series of six small test-tubes place increasing doses of the ricin or abrin solution as follows: 0.1, 0.2, 0.3, 0.4, 0.5, and 0.8 c.c. Add 1 c.c. of rabbit-cell emulsion to each and sufficient normal salt solution to make the total volume in each tube equal to 2 c.c. A seventh tube is the corpuscle control and contains 1 clc. of the erythrocyte suspension and 1 c.c. of salt solution.

6. Prepare a similar series of tubes with the guinea-pig erythrocyte suspension.

7. Shake the tubes gently and incubate for two hours.

(a) Do any of the tubes show hemolysis or hemagglutination?

(b) Is the action the same with both bloods?

(c) Does the plant toxin show a selective affinity?

(d) Does the rabbit show any evidences of hemolytic jaundice? Are there blood elements in the urine?

(e) Autopsy the animal, paying particular attention to the kidneys and gastro-intestinal tract? What changes have occurred?

EXPERIMENT 19.—ZOÖTOXIN (COBRA VENOM):

1. Collect about 2 c.c. of normal human blood and place in 5 c.c. of 2 per cent. sodium citrate in 0.85 per cent. sodium chlorid solution. This mixture must not be shaken and the cells should be washed at least four times with normal salt solution, after which they are made up to a 4 per cent. suspension.

2. Prepare a 2 per cent. suspension of sheep corpuscles in the same manner.

3. Prepare a stock 1 : 5000 dilution of cobra venom by dissolving 0.005 gm. dried venom in 10 c.c. of normal salt solution. This solution will keep about one week in the refrigerator.

4. Prepare a solution of venom 1 : 10,000 by diluting 2 c.c. of the stock solution with 8 c.c. salt solution. Prepare a 1 : 20,000 dilution by diluting 2 c.c. of dilution 1 : 10,000 with 2 c.c. salt solution.

5. Place 1 c.c. of corpuscle suspension into each of three small test-tubes and add 1 c.c. of each dilution of venom respectively. Shake the tubes gently and place in the incubator for an hour and then in the refrigerator overnight.

6. Repeat, using rabbit and guinea-pig corpuscles.

7. Inject 2 c.c. of the 1 : 5000 dilution intravenously into a rabbit and 1 c.c. intravenously into a guinea-pig.

8. Observe the animals closely, particularly the urine.

(a) Does the venom show a hemotoxic action? Does it act the same on rabbit and guinea-pig corpuscles? If not, why not?

(b) How do you explain venom hemolysis?

(c) What are the evidences of venom hemolysis *in vivo*?

(d) Is the hemotoxic poison the most dangerous constituent of venom?

ENDOTOXINS AND AGGRESSINS

EXPERIMENT 20.—ENDOTOXINS AND ARTIFICIAL AGGRESSINS:

1. Inoculate eight agar slants with *Bacillus typhosus* and cultivate at 37° C. for seventy-two hours.
2. Wash off the growths with 3 c.c. distilled water for each tube.
3. Place the emulsion in a sterile bottle with glass beads and shake for twenty-four hours at room temperature. At the same time inoculate six more slants of agar and wash off the growths at the end of twenty-four hours with 3 c.c. of normal salt solution for each tube.
4. Filter the emulsion, which has been shaken, through a Berkefeld or candle filter.
5. Inject two rabbits intravenously with 5 c.c. each of the filtrate (endotoxin) and unfiltered culture.
6. Observe influence on body weight and temperature and for symptoms of toxemia.
7. Inject three pigs intraperitoneally as follows:
 - First pig: 4 c.c. of emulsion of typhoid bacilli.
 - Second pig: 4 c.c. of filtrate.
 - Third pig: 2 c.c. each of emulsion and filtrate.

(a) Are there symptoms of toxemia in the rabbits? Are these symptoms alike in both animals? Which is more toxic, the filtrate or the whole culture?

(b) Does the filtrate enhance the effect of the whole culture?

(c) If any of the animals succumb, autopsy under aseptic precautions. Prepare cultures of the peritoneum and heart's blood on agar slants or in neutral bouillon. Is the typhoid bacillus markedly aggressive?

(d) If there is a peritoneal exudate, prepare smears and stain with carbolfuchsin (1 : 10). What type of cell predominates? Are any of the bacilli engulfed in the cells?

(e) Do any of the bacilli show signs of disintegration? If so, do you know what these changes are due to?

(f) Is the filtrate alone toxic?

(g) Does the filtrate appear to enhance the effects of the unfiltered culture?

BACTERIAL PROTEIN; PTOMAINS; MECHANICAL ACTION OF BACTERIA

EXPERIMENT 21.—BACTERIAL PROTEIN (VAUGHAN):

1. Inoculate 10 slants of 2 per cent. neutral agar in large bottles with a culture of *Bacillus coli* and grow at 37° C. for four days.
2. Add about 10 c.c. of distilled water to each bottle and gently remove the growth with a sterilized platinum wire, being particularly careful not to remove fragments of agar.
3. Pipet the heavy emulsion to a large centrifuge tube. Another cubic centimeter or two of water is added to each bottle to remove the balance of the growth.
4. Centrifuge at high speed until the bacilli are thoroughly settled. Decant off the supernatant fluid and add 50 per cent. alcohol; mix and centrifuge. Decant and add 95 per cent. alcohol; mix and centrifuge.
5. Remove the sediment of bacteria to a small flask with 50 c.c. of absolute alcohol and set aside at room temperature for a day. Decant off the alcohol and add 50 c.c. of ether. Mix and set aside for another twenty-four hours. Decant off the ether that remains and remove sediment to a porcelain or agate mortar. Place in the incubator for a few hours until thoroughly dry.
6. Grind the dry mass very thoroughly, the operator wearing a mask, until a fine powder is produced. Place the powder of bacterial substance in a wide-mouthed dark glass bottle and preserve in a dark closet. A portion will be needed later for experiments in anaphylaxis.
7. Mix 0.02 gm. of the dry powder with 10 c.c. salt solution.
8. Inject 2 c.c. of this emulsion intraperitoneally in a 300-gram guinea-pig.
9. Heat 2 c.c. of the emulsion at 60° C. for two hours and inject intraperitoneally in a guinea-pig.
10. Inject a third pig intraperitoneally with 2 c.c. of a four-day bouillon culture of *Bacillus coli*.

- (a) Could endotoxins withstand these various manipulations?
- (b) Does the heated extract kill the animal more quickly than the unheated, and if so, why?
- (c) What are the symptoms produced?
- (d) Autopsy the animals. Are there evidences of peritonitis? Are there differences in the lesions of the three animals? If so, how do you explain them?
- (e) The bacterial split portion will be studied later under Anaphylaxis.

EXPERIMENT 22.—PTOMAINS:

1. Procure 4 ounces of beef and mince.
 2. Place in a flask with 250 c.c. tap-water and inoculate with a culture of *Bacillus coli*.
Fit the flask with a rubber stopper with a glass tube to carry off gases.
 3. Inoculate a flask of neutral bouillon at the same time with the same culture.
 4. Cultivate both at 37° C. for several days.
 5. Filter both through a coarse Berkefeld filter.
 6. Concentrate both filtrates to one-half their volume at a low temperature on a water-bath.
 7. Inject two guinea-pigs with each preparation, giving 10 c.c. subcutaneously and 5 c.c. intraperitoneally.
 8. Observe the four animals closely.
- (a) What symptoms develop in both sets of animals?
 - (b) What are ptomains? What rôle do they play in the production of disease?
 - (c) Can antibodies be produced for true ptomains?

EXPERIMENT 23.—MECHANICAL ACTION OF BACTERIA:

1. Inject a 300-gram pig intraperitoneally with 5 c.c. of a forty-eight-hour-old bouillon culture of virulent *Bacillus anthracis*. Great care must be exercised in handling and injecting this culture.
 2. Autopsy the animal at the end of forty-eight hours and make cultures on agar slants of the heart's blood, liver, and spleen. Remove the heart, lungs, liver, spleen, and kidneys, and place in 2 per cent. formalin. Prepare smears of the blood and stain with Gram's method.
 3. After fixing for twenty-four hours, sections of these organs are to be prepared and stained with methylene-blue and with Gram's stain.
 4. Examine the cultures at the end of twenty-four hours.
 5. Examine the sections.
- (a) Is the anthrax bacillus aggressive?
 - (b) Does the anthrax bacillus produce much toxin?
 - (c) Did the animal show any symptoms of its infection?
 - (d) Are there evidences of peritonitis?
 - (e) How do you explain the probable causes of death in human anthrax?

KINDS OF IMMUNITY

EXPERIMENT 24.—PHAGOCYTOSIS IN NATURAL IMMUNITY:

1. Inject a healthy guinea-pig intraperitoneally with 1 c.c. of a twenty-four-hour or forty-eight-hour glucose bouillon culture of streptococci of low or moderate virulence. A culture of staphylococci may be used instead, although the former is preferable.
 2. At the end of eighteen hours study the peritoneal fluid. Prepare smears and stain with methylene-blue or other suitable stain. Prepare cultures in glucose bouillon of the heart's blood.
- (a) Did the animal show any evidences of infection?
 - (b) Has phagocytosis occurred in the peritoneal cavity?
 - (c) What constituent of normal serum aids phagocytosis?
 - (d) Did a bacteremia develop?

EXPERIMENT 25.—NATURAL ANTIBACTERIAL IMMUNITY:

1. Inject a 250-gram guinea-pig subcutaneously with 5 c.c. of a twenty-four-hour bouillon culture of *Bacillus anthracis*.
2. Inject a large albino rat with the same dose and in the same manner.
3. Observe the animals for twenty-four to forty-eight hours. At autopsy prepare smears and cultures of the heart blood of each. Stain the smears with methylene-blue and according to the method of Gram.

(a) Do the animals present symptoms of infection? Are there any differences between the two?

(b) Are anthrax bacilli found in the smears and cultures of both animals? Which animal is immune? Could phagocytosis play a rôle in this immunity?

EXPERIMENT 26.—RELATIVE FACTORS IN NATURAL IMMUNITY:

1. Place a frog in a shallow vessel of warm water in the incubator at 37° C.
2. After twenty-four hours give a subcutaneous injection of tetanus toxin equal to one-tenth the fatal dose for a 300-gram guinea-pig.
3. Inject a second frog with an equal amount of toxin and keep it at ordinary room temperature in cold water.
4. Observe both animals.

(a) Do symptoms of tetanus develop in any of the animals?

(b) Why does heat favor tetanus infection?

(c) Give further examples of relative natural immunity.

EXPERIMENT 27.—INFLUENCE OF TEMPERATURE UPON NATURAL IMMUNITY:

1. Procure dried tetanus toxin and dissolve sufficient in 6 c.c. normal salt solution equivalent to 6 lethal doses for a 350-gram guinea-pig.
2. Inject 2 c.c. into the pectoral muscles of a young hen. Take the rectal temperature.
3. Inject 2 c.c. into a second hen. Take the rectal temperature and place her in cold water until the temperature has dropped several degrees.
4. Place hen No. 1 in a cage and keep her at ordinary laboratory temperature.
5. Place hen No. 2 in a cage and keep her in a cold place or renew the bath several times in order to keep her temperature subnormal.

(a) What is the normal temperature of the hen?

(b) Do both animals present symptoms of tetanus?

(c) How do you explain the difference?

6. If hen No. 1 does not show symptoms of tetanus by the second day, reinject her with an amount of toxin equal to 10 lethal doses for a guinea-pig.

(a) Does this hen present evidences of tetanus?

(b) If so, how do you explain the result?

EXPERIMENT 28.—NATURAL IMMUNITY TO DIPHTHERIA:

1. Secure diphtheria toxin in which the M. L. D. and L+ doses are known.
2. Inject each of two white rats intraperitoneally with 1000 M. L. D. Inject a third rat with 10,000 M. L. D.
3. Next morning bleed one of the first two rats and inject the serum into a guinea-pig (intraperitoneally).
4. Dilute a portion of the toxin with saline solution in such manner that 0.2 c.c. carries 1/50 of the L+ dose.
5. Secure blood from additional normal rats and defibrinate; centrifuge and secure the serum.
6. In a small test-tube place 0.2 c.c. of the diluted toxin and 0.8 c.c. of the serum; in a second tube place 0.2 c.c. of the toxin and 0.8 c.c. of saline solution. Allow mixtures to stand thirty minutes.
7. Secure a 10- to 12-ounce guinea-pig; pluck the hairs away at three places on the abdomen. Inject 0.1 c.c. of each of the three mixtures *intracutaneously*.
8. Observe all of the animals daily for evidences of inflammatory reactions and necroses.

- (a) Is the rat naturally immune to diphtheria toxin? Is the guinea-pig?
- (b) Is the immunity of the rat relative or absolute?
- (c) Is natural diphtheria antitoxin present in rat serum?
- (d) Upon what does the natural immunity of the rat to diphtheria depend?

EXPERIMENT 29.—NATURAL IMMUNITY TO DIPHTHERIA; THE SCHICK TEST:

1. Secure diphtheria toxin of known titer and dilute for the Schick test as described in the text.
2. Inject the proper amount *intracutaneously*; it is well for each member of the class to take an injection.
3. Also secure the control fluid and inject *intracutaneously*.
4. Read the reactions at the end of twenty-four and forty-eight hours; if positive make daily observations on the course and gradual subsidence of the reaction.

- (a) To what is natural immunity of human beings to diphtheria toxin due?
- (b) Discuss the relation of age to the Schick reaction.
- (c) Describe the appearance, mechanism, and significance of the positive reaction; the negative reaction.
- (d) Discuss the appearance, mechanism, and significance of the pseudo-positive reaction.
- (e) Discuss the practical value of the Schick test.

ACQUIRED IMMUNITY

EXPERIMENT 30.—ACQUIRED ACTIVE (ANTIBACTERIAL) IMMUNITY:

1. Immunize a rabbit with typhoid bacilli.
 2. Inject this immune rabbit intravenously with 6 loopfuls of a twenty-four-hour culture of *Bacillus typhosus* thoroughly emulsified in 2 c.c. sterile salt solution.
 3. Inject a normal rabbit of equal weight with an equal dose and in the same manner.
 4. Both animals are weighed and their temperatures recorded before the injections are given. If possible, take temperature every four hours. Observe both animals for symptoms of infection. If one or both succumb, autopsy aseptically and culture the heart blood in neutral bouillon or bile.
- (a) Are there differences in the clinical condition of the two animals?
 - (b) To what do you ascribe these differences?
 - (c) What chief antibody is responsible for the destruction of the bacilli in the immune animal?
 - (d) Has typhoid bacteremia developed?

PASSIVE ACQUIRED IMMUNITY

EXPERIMENT 31.—ACQUIRED PASSIVE (ANTITOXIC) IMMUNITY:

1. Inject a 250- to 300-gram guinea-pig subcutaneously with 1 c.c. (500 units) of diphtheria antitoxin (pig No. 1).
 2. Secure diphtheria toxin in which the L+ dose is known.
 3. Place this dose of toxin in a small test-tube or, better, in the barrel of a precision syringe (Hitchens), and add 1 c.c. antitoxin (500 units). Mix thoroughly and keep at room temperature for an hour.
 4. Inject pig No. 1 with an amount of toxin equal to the L+ dose.
 5. Inject the toxin and antitoxin mixture into a second pig of 250 to 300 grams.
 6. Inject the same amount of toxin into a third pig of equal weight as a control.
 7. Observe animals closely for at least four or five days.
- (a) What are the chief symptoms of diphtheria intoxication of the guinea-pig? What are the chief pathologic changes?
 - (b) Do all animals show these symptoms?
 - (c) How do you explain the absence of symptoms in pig No. 1? How in pig No. 2? Is the mechanism of protection the same in both?

(d) What bearing has this experiment upon the treatment of diphtheria?

EXPERIMENT 32.—ACQUIRED PASSIVE (ANTITOXIC) IMMUNITY:

The above experiment may be conducted in exactly the same manner, using tetanus toxin and antitoxin.

EXPERIMENT 33.—ACQUIRED PASSIVE (ANTIBACTERIAL) IMMUNITY:

1. Secure a culture of Type I pneumococcus, and if its lethal dose for white mice is unknown, determine this by injecting a series of mice with decreasing doses of a twenty-four-hour serum bouillon culture.

2. Secure a few cubic centimeters of Type I antipneumococcus serum.

3. Inject three mice intraperitoneally with 0.1, 0.5, and 1 c.c. of the serum. Then inject them intraperitoneally with 1000 minimal lethal doses of pneumococci. Inject a fourth mouse with culture alone (control).

4. Observe the animals for several days, and at autopsy culture the heart blood in tubes of serum bouillon and prepare smears which are to be stained according to Gram.

(a) Has the serum served to protect any of the mice?

(b) Is this protection due to bacteriolysins, bacteriotropins, or both? Do antitoxins play any part?

(c) Why is it preferable to use homologous culture and immune serum? What bearing has this upon the treatment of human pneumococcus infections?

(d) Do you know a method for quickly isolating and identifying pneumococci from sputum?

Note.—This experiment may be conducted with rabbits; also a culture of streptococcus and its immune serum may be used.

PHAGOCYTOSIS AND CHEMOTAXIS

EXPERIMENT 34.—PHAGOCYTOSIS (MACROPHAGES):

1. Secure pigeons' blood and defibrinate. Wash the corpuscles several times and prepare a 5 per cent. suspension.

2. Inject a guinea-pig intraperitoneally with 3 c.c. of this corpuscle suspension.

3. After three hours withdraw a small amount of peritoneal exudate by means of a capillary pipet. Examine with hanging-drop preparations and prepare smears and stain with Wright's stain.

4. Make similar preparations twelve, eighteen, twenty-four, and forty-eight hours after injection.

(a) Has phagocytosis occurred?

(b) Which cells have become phagocytes?

(c) Do the pigeon cells appear as if undergoing digestion?

(d) Which portion of the pigeon cell resists digestion?

(e) Explain mechanism of intracellular digestion.

EXPERIMENT 35.—PHAGOCYTOSIS (MICROPHAGES):

1. Place a drop of blood in the hollow cell of a ground-out slide such as is used for hanging-drop preparations. Cover with a clean slide, seal with a ring of vaselin, and place in a large Petri dish containing pieces of filter-paper moistened with water (moist chamber). Place in the incubator for fifteen minutes.

2. Remove the cover-glass and carefully wash cover-glass and cell with normal salt solution. The erythrocytes are removed and the leukocytes left adherent to the cell and cover-glass.

3. Fill the cell with fresh serum and add a quantity of culture, preferably a loopful of a twenty-four-hour bouillon culture of non-virulent anthrax bacilli. Apply the cover-glass and vaselin the margins to prevent evaporation.

4. If at all possible, employ a warm stage and watch the process under the microscope.

- (a) Does phagocytosis occur?
- (b) Which cells are acting as phagocytes?
- (c) Can you make out the phase of fixation and phase of ingestion?
- (d) By what agencies do leukocytes kill engulfed bacteria?

EXPERIMENT 36.—PHAGOCYTOSIS; POSITIVE CHEMOTAXIS:

1. Inject a guinea-pig intraperitoneally with 5 c.c. of finely divided cinnabar suspension and 1 c.c. subcutaneously on each side in the region of the inguinal lymph-glands.

2. Autopsy the animal at the end of twenty-four hours. Prepare hanging-drop preparations and smears of the peritoneal exudate (stained lightly with methylene-blue). Prepare sections of the inguinal and abdominal lymphatic glands.

- (a) Has phagocytosis occurred in the peritoneal cavity? Which cells have become phagocytic?
- (b) Do you find phagocytes in the lymph-glands? Where are the leukocytes situated? Which cells have become phagocytes?
- (c) How did the material reach the glands?
- (d) How do you explain the presence of cinnabar in the endothelial cells of the gland?

1. Inject a guinea-pig intraperitoneally with 10 c.c. of a twenty-four-hour culture of *Staphylococcus aureus*.

2. Autopsy the animal eighteen hours later and prepare smears of the exudate. Fix with methyl alcohol for five minutes, dry, and stain with carbol-thionin, Wright's, or Löffler's methylene-blue, counterstained with eosin.

3. Prepare cultures of the peritoneal exudate.

- (a) Describe the exudate.
- (b) Has phagocytosis occurred?
- (c) Which cells are actively phagocytic?
- (d) Are all the cocci engulfed?
- (e) Are there any evidences of the cocci undergoing intracellular digestion?
- (f) Could a sterile substance call forth an exudation of leukocytes when injected into a serous cavity?

EXPERIMENT 37.—NEGATIVE CHEMOTAXIS:

1. Inject a guinea-pig intraperitoneally with 5 c.c. of a forty-eight-hour serum bouillon culture of *virulent* streptococci.

2. Autopsy at the end of eighteen to twenty-four hours if death has not already occurred.

3. Prepare cultures in serum bouillon and a number of smears. Stain the latter with methylene-blue or according to Gram.

- (a) Describe the exudate. How does it differ from that found in the preceding experiment?
- (b) How do you explain the serous character of the exudate?
- (c) Has phagocytosis occurred?
- (d) Do you think the streptococci have multiplied in the peritoneal cavity?
- (e) What means could you suggest for overcoming this action of virulent streptococci?

OPSONINS

EXPERIMENT 38.—NORMAL OPSONINS:

1. Prick the finger and secure 1 c.c. of blood in a small test-tube. Also 1 c.c. in a centrifuge tube containing 2 c.c. of sodium citrate solution. After coagulation remove the serum from the first tube.

2. Divide the serum into two portions and heat one portion at 56° C. for thirty minutes.
3. Prepare an emulsion of leukocytes by centrifuging the blood collected in the citrate solution, removing the supernatant fluid, adding normal salt solution, and centrifuging again. Repeat this step once more in order to wash the cells thoroughly and after the last centrifuging remove the supernatant fluid and add *sufficient salt solution to make the total volume 1 c.c. and mix thoroughly.*
4. Prepare an emulsion of staphylococci which is homogeneous and free of clumps.
5. Mark two capillary tubes with a wax pencil about 1 inch from the tip; fit rubber teats to the other end.
6. With pipet No. 1 take up a volume of blood-cells; allow a bubble of air to enter and then an equal volume of bacterial emulsion; bubble of air and an equal volume of the **fresh** unheated serum. Mix well by alternate expulsion and aspiration on a clean slide. Then draw the whole into the stem of the pipet and seal the tip in a flame.
7. Repeat with pipet No. 2, using the heated serum.
8. Incubate both pipets at 37° C. for half an hour.
9. Remove the pipets from the incubator, break off the tips, mix the contents, and prepare smears.
10. Fix the smears with a saturated solution of bichlorid of mercury for one minute; wash in water and stain with carbol-thionin for two minutes; wash in water and dry.
11. Examine with oil-immersion lens.

- (a) What are the requisites of a satisfactory opsonic preparation?
- (b) Is there any difference in the amount of phagocytosis between the heated and unheated serums? If so, how do you explain the result?
- (c) Do normal opsonins play any rôle in natural immunity?
- (d) Give the properties of normal opsonins.

EXPERIMENT 39.—IMMUNE OPSONINS (BACTERIOTROPINS):

1. Secure 1 c.c. of serum from a rabbit immunized with staphylococci and heat 0.5 c.c. at 56° C. for thirty minutes.
 2. Using the same blood and bacterial emulsion as prepared in the preceding experiment prepare two opsonic preparations with the heated and unheated immune serum.
- (a) Is phagocytosis more marked than in the preceding experiment? If so, why?
 - (b) Is there any difference in the degree and extent of phagocytosis with the heated and unheated serum?
 - (c) Give the properties of immune opsonin or bacteriotropin.

EXPERIMENT 40.—QUANTITATIVE ESTIMATION OF BACTERIOTROPINS:

1. Secure a culture of normal meningococci and a small amount of polyvalent antimeningococcus serum.
 2. Determine the tropin titer according to the technic described in the text.
- (a) Define the meaning of opsonic index versus phagocytic index.
 - (b) Discuss the relation of bacteriotropins to mechanism of therapeutic activity of antimeningococcus sera.
 - (c) Define opsonins versus bacteriotropins.
 - (d) What relation do bacteriotropins bear to immunity?
 - (e) In what diseases are bacteriotropins active?

EXPERIMENT 41.—HEMOPSONIN:

1. Secure 0.5 c.c. serum from a rabbit immunized with sheep cells and heat at 56° C. for a half an hour to destroy hemolytic complement.
2. Prepare a 5 per cent. suspension of sheep erythrocytes in normal salt solution after washing them three times.
3. Prepare an emulsion of rabbit leukocytes; the emulsion prepared in the preceding experiments may be used.
4. Take a capillary pipet; make a mark about 1 inch from the tip and fit the other end with a rubber teat.

5. Draw up equal volumes of leukocytic emulsion, sheep cell suspension, and serum. Mix well, seal the pipet, and inoculate for an hour at 37° C.
6. Prepare smears and stain with Wright's blood-stain.

- (a) Has phagocytosis occurred?
- (b) Which cells have become phagocytes?
- (c) Has hemolysis occurred in the mixtures, and if not, why not?

EXPERIMENT 42.—MECHANISM OF ACTION OF OPSONINS AND BACTERIOTROPINS:

1. Secure serum from a rabbit immunized with *Staphylococcus pyogenes aureus*.
2. Prepare a suspension of rabbit leukocytes.
3. Prepare an emulsion of eighteen-hour culture of *Staphylococcus aureus*.
4. Make the following mixtures in capillary pipets:
 - No. 1: Equal parts of leukocytes, serum, and bacterial emulsion.
 - No. 2: Equal parts of leukocytes and bacterial suspension.
5. In two small test-tubes mix:
 - No. 3: 0.5 c.c. each of leukocytic suspension and serum.
 - No. 4: 0.5 c.c. each of serum and bacterial emulsion.
6. Incubate pipets 1 and 2 and tubes 3 and 4 for fifteen minutes at 37° C.
7. Prepare smears of capillary tubes 1 and 2 and stain with carbol-thionin.
8. To tubes 3 and 4 add 15 c.c. normal salt solution, mix, and centrifuge thoroughly. Decant off the supernatant fluid and restore volume in each tube to 1 c.c.
9. Prepare capillary pipets as follows:
 - No. 3: Equal parts of contents of tube 3 and bacterial emulsion.
 - No. 4: Equal parts of contents of tube 4 and leukocytic mixture.
10. Incubate both pipets for fifteen minutes, prepare smears, and stain with carbol-thionin.

(a) Carefully compare the degree and extent of phagocytosis in the four mixtures.

(b) Has leukocytosis occurred in mixture No. 2? If so, what special term is applied to phagocytosis in the absence of serum?

(c) What rôle does serum play in phagocytosis? Explain the mechanism involved.

(d) Discuss the question of "stimulin" and opsonin as demonstrated by the results in preparations Nos. 3 and 4.

EXPERIMENT 43.—SPECIFICITY OF OPSONINS AND BACTERIOTROPINS:

1. Secure 1 c.c. of the serum of a rabbit immunized with staphylococci and heat at 56° C. for thirty minutes. Divide into two portions in separate small test-tubes.
2. To No. 1 add 1 c.c. of normal salt solution and 4 or 5 loopfuls of a twenty-four-hour agar slant culture of staphylococci. Incubate for thirty minutes; centrifuge thoroughly and remove the supernatant fluid (diluted serum) to a separate tube. Call this "treated" serum.
3. To the untreated serum add 1 c.c. salt solution, so that both are diluted equally.
4. Prepare an emulsion of rabbit or human leukocytes.
5. Prepare an emulsion of staphylococci, homogeneous and free of clumps.
6. Prepare two opsonic mixtures: No. 1 containing equal parts of blood suspension, bacterial emulsion, and untreated serum; No. 2 containing equal parts of blood, bacterial emulsion, and treated serum.
7. Incubate for thirty minutes. During this interval proceed as follows:
8. Prepare an emulsion of typhoid bacilli, homogeneous and free of clumps.
9. Secure 0.5 c.c. of serum from a rabbit immunized with typhoid bacilli and heat at 36° C. for thirty minutes.
10. Prepare the following mixtures in capillary pipets:
 - No. 3 blood-cells + typhoid serum + typhoid bacterial emulsion.
 - No. 4 blood-cells + typhoid serum + staphylococcus bacterial emulsion.
 - No. 5 blood-cells + staphylococcus serum + typhoid emulsion.
11. Incubate for fifteen minutes.
12. Prepare smears of all and stain with carbol-thionin.

(a) Is there any difference in the degree of phagocytosis in mixtures No. 1 and 2? If so, why?

(b) Are opsonins and bacteriotropins specific?

(c) Has phagocytosis occurred in the cross-mixtures of staphylococci with typhoid serum, and typhoid bacilli with staphylococcus serum? If so, how do you explain this apparent lack of specificity?

(d) What may happen in a mixture of fresh unheated typhoid serum, typhoid bacilli, and leukocytic emulsion?

OPSONIC INDEX

EXPERIMENT 44.—DETERMINING THE OPSONIC INDEX:

1. Secure a small quantity of blood from a guinea-pig or rabbit which has been immunized with *Staphylococcus pyogenes aureus*. Also two specimens from normal animals to serve as control serums. Remove the serums, being careful to keep them marked and separate. The two normal serums may be mixed in a watch-glass or small test-tube (pooled).

2. Prepare a leukocyte mixture, using normal guinea-pig or rabbit blood according to the immune animal used.

3. Prepare a bacterial emulsion of an eighteen-hour culture of *Staphylococcus pyogenes aureus*.

4. Proceed to determine the phagocytic and opsonic index as per technic given in the text.

(a) What constitutes a satisfactory bacterial emulsion?

(b) What constitutes a satisfactory leukocytic suspension? Name several methods of obtaining leukocytes for this technic.

(c) What constitutes a satisfactory phagocytic film?

(d) Should the serum be fresh?

(e) How do you determine the phagocytic index?

(f) How do you determine the opsonic index?

(g) What is the relation between the opsonic and phagocytic indices?

(h) Give the practical value of the opsonic index in disease.

(i) Give the practical value of the opsonic index as a measure of immunity.

Note.—If any of the students have received typhoid vaccine, the opsonic index with his serum may be determined.

BACTERIAL VACCINES

EXPERIMENT 45.—PREPARATION OF TYPHOID VACCINE :

1. Prepare two to six agar slant cultures of *Bacillus typhosus* and grow for forty-eight hours at 37° C.

2. Remove cultures, prepare a suspension, count by the method of Wright, sterilize, dilute, and prepare vaccine for administration as given in the text.

3. Prepare two emulsions: one to contain about 500,000,000 bacilli in each cubic centimeter (first dose), and the second 1,000,000,000 per cubic centimeter (second and third doses).

EXPERIMENT 46.—PREPARATION OF STAPHYLOCOCCUS VACCINE:

1. Prepare two to six agar slant cultures of *Staphylococcus aureus* and grow for twenty-four hours at 37° C. If a patient with furunculosis is available, make cultures of pus and secure staphylococcus, of which a vaccine is prepared.

2. Proceed in the preparation of the vaccine as given in the text, placing each dose in separate ampules, and so diluting that each dose is of 1 c.c. and contains 1,000,000,000 of cocci. Count by the method of Wright and by the counting chamber method.

ANTITOXINS

EXPERIMENT 47.—STANDARDIZING DIPHTHERIA ANTITOXIN:

1. Prepare a strong diphtheria toxin with the Park-Williams *Bacillus* No. 8, after the technic given in the text.

2. Secure some of the dried Standard Antitoxin and dilute so that 1 c.c. is equal to one immunity unit.

3. With this antitoxin determine the L+ dose of the toxin prepared according to the technic given, using 6 guinea-pigs and the following doses of toxin: 0.1, 0.12, 0.15, 0.18, 0.2, and 0.25 c.c.

4. Secure a sample of diphtheria antitoxin in the open market containing about 4 c.c. serum and 2000 units of antitoxin. If the titration given on the label is still correct, one would expect about 500 units of antitoxin per cubic centimeter of serum. Carefully remove 1 c.c. of serum and dilute with 19 c.c. salt solution (1:20). From this stock dilution prepare the following dilutions (taken from Bulletin No. 21, Hygienic Laboratory, M. J. Rosenau):

1 c.c. + 14 c.c. NaCl solution	1 c.c. = .00333 or 1/300 = 300 units per c.c.
1 c.c. + 16 c.c. NaCl solution	1 c.c. = .00294 or 1/340 = 340 units per c.c.
1 c.c. + 18 c.c. NaCl solution	1 c.c. = .00263 or 1/380 = 380 units per c.c.
1 c.c. + 20 c.c. NaCl solution	1 c.c. = .00238 or 1/420 = 420 units per c.c.
1 c.c. + 22 c.c. NaCl solution	1 c.c. = .00217 or 1/460 = 460 units per c.c.
1 c.c. + 24 c.c. NaCl solution	1 c.c. = .002 or 1/500 = 500 units per c.c.

5. Mix 1 c.c. of these various dilutions with the L+ dose of toxin; stand aside for an hour and inject subcutaneously in median abdominal line of 250- to 300-gram guinea-pig as per the technic already given.

6. Carefully observe all animals for a period of four days at least. Autopsy those that succumb, paying particular attention to the condition of the abdominal wall and suprarenal glands. If the serum should contain less than 300 units of antitoxin per cubic centimeter of serum, the test should be repeated with lower dilutions.

7. Inject 1 c.c. of the serum subcutaneously into a white mouse to test for excess of preservative. It requires 1 c.c. of a 0.5 per cent. solution of tricresol or 0.5 c.c. of a 0.5 per cent. phenol solution to kill a medium-sized mouse. If the mouse shows trembling, it would indicate that the serum contains nearly this percentage of tricresol (Bulletin No. 21, Hygienic Laboratory).

8. Inoculate 1 c.c. of the serum in a flask containing 100 c.c. sterile neutral bouillon. Incubate at 37° C. for at least four days. This will test the sterility of the product.

(a) Define the unit of diphtheria antitoxin.

(b) Of what practical value is the measurement of diphtheria antitoxin?

(c) Is there any practical method of determining the quantity of toxin in the blood of a diphtheric patient?

(d) How would you determine the amount of natural antitoxin in the blood of a person?

EXPERIMENT 48.—STANDARDIZING TETANUS ANTITOXIN:

The technic of standardizing tetanus antitoxin may be carried out in a similar manner with dried Standard Toxin and an antitoxin purchased in the open market.

EXPERIMENT 49.—SPECIFICITY OF ANTITOXINS:

1. Secure small quantities of fresh diphtheria and tetanus toxins; the L+ dose of each should be known.

2. Secure small quantities of diphtheria and tetanus antitoxins; the number of units per cubic centimeter of serum should be known.

3. Into four precision syringes place the following mixtures. After standing an hour at room temperature, inject subcutaneously into 300-gram guinea-pigs.

No. 1: L+ dose of diphtheria toxin + 100 units of diphtheria antitoxin. Inject into pig No. 1.

No. 2: L+ dose of diphtheria toxin + 100 units of tetanus antitoxin. Inject into pig No. 2.

No. 3: L+ dose of tetanus toxin + 100 units of tetanus antitoxin. Inject into pig No. 3.

No. 4: L+ dose of tetanus toxin + 100 units of diphtheria antitoxin. Inject into pig No. 4.

(a) What do you observe regarding the specificity of antitoxins?

(b) What are the main symptoms of diphtheria and tetanus in the guinea-pig?

(c) Even though a pig injected with diphtheria toxin shows no general symptoms of intoxication, what local sign may be present?

(d) What is the nature of the toxin-antitoxin reaction?

EXPERIMENT 50.—NATURE OF THE TOXIN-ANTITOXIN REACTION. ACTION OF ANTITETANOLYSIS:

1. Secure fresh tetanus toxin and determine the dose producing complete hemolysis of 1 c.c. of a 1 per cent. suspension of rabbit corpuscles in two hours at 37° C.

2. Place double this dose of toxin in a series of six small test-tubes and add increasing doses of fresh tetanus antitoxin: 0.001, 0.005, 0.01, 0.05, 0.1, and 0.2 c.c. Add salt solution to bring the total volume to 1 c.c.; incubate at 37° C. for one hour. Add of 1 per cent. suspension of rabbit corpuscles to each tube. Prepare two controls, one with the dose of toxin and corpuscles, but to which no serum is added, the second with 0.2 c.c. serum and dose of corpuscles. Shake tubes and incubate for two hours. Make a preliminary reading and again after tubes have settled twenty-four hours in the refrigerator.

3. The first control should be completely hemolyzed, indicating that a sufficient lytic dose of toxin was employed.

(a) What constituent of tetanus toxin has a marked affinity for erythrocytes?

(b) Is this agent thermostabile? How can you determine this?

(c) What rôle does it play in tetanus intoxication?

(d) How is this hemotoxic agent neutralized by tetanus antitoxin?

(e) Would anti-tetanospasmin neutralize the hemotoxic activity of tetanus toxin?

(f) Would diphtheria antitoxin neutralize this hemotoxic agent? If not, why not?

(g) Explain the mechanism of neutralization of a toxin by antitoxin *in vitro*. Is it the same as that occurring *in vivo*?

EXPERIMENT 51—ANTISTAPHYLOLYSIN:

1. Prepare a staphylolysin by growing a culture of *Staphylococcus aureus* in bouillon for two or three days. Pass through a Berkefeld filter and preserve the filtrate with 0.5 phenol. Determine the lytic dose for 1 c.c. of a 1 per cent. suspension of rabbit corpuscles.

2. Secure serum from a rabbit immunized with staphylococci. Heat at 56° C. for thirty minutes.

3. Secure normal horse-serum. Heat it at 56° C. for thirty minutes.

4. Secure normal rabbit-serum. Heat at 56° C. for thirty minutes.

5. Into a series of six small test-tubes place the lytic dose of staphylolysin and increasing amounts of rabbit immune serum as follows: 0.001, 0.005, 0.01, 0.05, 0.1, 0.2 c.c. Arrange a similar series, using normal horse-serum and normal rabbit-serum. Prepare a control containing the lytic dose of toxin. Add 1 c.c. of a 1 per cent. suspension of rabbit cells to each tube and sufficient normal salt solution to make the total volume equal 2 c.c. Shake each tube gently and incubate at 37° C. for two hours.

6. Inspect the tubes. The smallest amount of normal horse-serum inhibiting hemolysis is taken as 1, or the unit. Compare the values of the immune and normal rabbit-serums with this unit.

(a) Why is normal horse-serum adopted as the standard?

(b) What is antistaphylolysin?

(c) What are the various agents produced by staphylococci and responsible for the lesions and symptoms of staphylococcus infections?

(d) Would the antilysin neutralize the leukocidin?

(e) Explain the mechanism of lysin-antilysin action.

(f) Which rôle does the lysin play in staphylococcus infections?

(g) Of what value would be the titration of antistaphylolysin in the serum of the patient?

FERMENTS AND ANTIFERMENTS**EXPERIMENT 52.—TRYPTIC FERMENT OF LEUKOCYTES:**

1. Collect 0.5 c.c. of blood in each of two small test-tubes by puncture of a finger; set aside until coagulation has occurred. Add several changes of warm distilled water until the red corpuscles are hemolyzed and colorless clots of leukocytes, fibrin platelets, and detritus are secured.

2. Place one clot in the bottom of a tube of sterile and slanted Löffler blood-serum medium which is fairly dry and firm.

3. Place the second clot in a second tube of this medium and add 0.2 to 0.4 c.c. of fresh serum.

4. Plug these tubes firmly, paraffin the stoppers to prevent evaporation, and incubate along with a non-inoculated control at 50° C. for two days.

(a) In which tube do you note evidences of digestion?

(b) Describe the appearance of the digested medium.

(c) Does the tube containing serum show digestion? How do you explain the result?

(d) What rôle may this ferment play in infection?

(e) Why do not the leukocytes digest themselves?

(f) What is the nature of the ferment?

(g) May this ferment play a rôle in the disposal of old and dead leukocytes?

EXPERIMENT 53.—TESTING THE ANTITRYPTIC POWER OF BLOOD-SERUM (AFTER THE MARCUS MODIFICATION OF THE METHOD OF MÜLLER AND JOCHMANN):

1. Prepare a solution of trypsin by thoroughly shaking 0.1 gm. of Kahlbaum's trypsin with 5 c.c. glycerin and 5 c.c. distilled water. Incubate at 55° C. for half an hour, shake thoroughly, filter, and preserve in the refrigerator.

2. Secure six Petri dishes of Löffler blood-serum culture-media which have been sufficiently dried to drive off the water of condensation and with a firm elastic surface.

3. Collect 1 c.c. of blood from a patient three hours after last meal; separate the serum.

4. In a watch-glass or hanging-drop slide mix 1 drop of serum with an equal sized drop of trypsin solution. Mix well and transfer five loopfuls to an area on a Petri dish of medium. With a blue-wax pencil draw a circle on the cover of the plate to include the site of planting and mark No. 1.

5. Prepare serial dilutions with 1 drop of serum with 2, 3, 4, 5, 6, 7, 8, 9, and 10 drops of trypsin solution. Mix well and plant five loopfuls of each dilution on the medium in five dishes. Two plantings may be made in each plate and with care they will not become confluent. Mark each plate.

6. Inoculate the sixth plate with 5 drops of trypsin solution without serum (control).

7. Incubate all tubes at 37° C. for six to twelve hours until the control shows well-marked digestion.

8. Wherever the trypsin has not been neutralized by the serum, shallow liquefied dimples appear on the surface of the Löffler medium. The greater the amount of trypsin required to cause digestion, the higher the titer of antitrypsin in the blood. By conducting a control series with the two normal pooled serums one may determine whether in a given case the antitryptic power of the blood is normal, increased, or decreased.

(a) Does this test possess any practical value?

(b) Discuss the presence of antitrypsin in the blood-serum.

BACTERIAL AGGLUTININS

EXPERIMENT 54.—THE GRUBER-WIDAL REACTION IN TYPHOID FEVER ("WET" METHOD). MICROSCOPIC REACTION:

1. Collect blood in a Wright capsule or small test-tube from a typhoid convalescent patient. Rabbit immune serum may be used instead. Separate the serum from the clot.

2. Prepare two dilutions with hanging-drop slides, 1 : 50 and 1 : 100, and a culture control. Use a twenty-four-hour bouillon culture of *Bacillus typhosus*—one free of clumps and in which the bacilli are long and motile.

3. It is well to prepare a similar test with a known positive typhoid serum and a normal negative serum.

4. Dilution and slides are prepared according to the technic given in the text. A second set of tests in dilutions of 1 : 40 and 1 : 80 may be prepared with the aid of the white corpuscle pipet.

5. Place slides away from direct sunlight and examine at the end of an hour. Examinations are readily made with the $\frac{1}{2}$ objective, the light being well cut off. Examine the culture control first, then the higher and lower dilutions.

- (a) Describe the phenomenon of agglutination.
- (b) Is it necessary for all bacilli to be agglutinated to constitute a positive reaction?
- (c) What are the features of a doubtful reaction? Of a negative reaction?
- (d) Does the normal serum contain agglutinin for the typhoid bacillus?
- (e) Why is it necessary to use high dilutions? What dilution is the lower limit of practical safety?
- (f) Does the control show agglutination? If so, by what term is this agglutination known?
- (g) What are the characteristics of a satisfactory culture for the microscopic agglutination test?
- (h) Would a dead culture be serviceable in this reaction?
- (i) What practical value has the Widal reaction in the diagnosis of typhoid fever?
- (j) What value has the agglutination reaction in determining the degree of immunity following typhoid immunization?

Note.—If the students are immunized with typhoid vaccine, they may use one another's serum in this and following experiments.

EXPERIMENT 55.—GRUBER-WIDAL REACTION IN TYPHOID FEVER ("DRY" METHOD):

1. Prepare smears of blood of a typhoid convalescent patient on clean glass slides or collect a few drops on partially glazed paper, as prescription blanks. Allow blood to dry and do not apply heat.
2. Using a good twenty-four-hour-old culture of *Bacillus typhosus*, prepare an agglutination test after the technic given in the text. Be particularly careful not to transfer paper fiber to the slide—apply the salt solution and dissolve the blood by gently rubbing with a small platinum loop (2 mm.). Mix the blood in the loopful of culture with the slide held or placed over a white surface so that the proper delicate orange tint is secured.
3. Prepare culture control as usual; also similar tests with a known positive and negative serum.
4. Examine at the end of an hour.
 - (a) What special precautions are to be observed in this method?
 - (b) What is the particular practical value of this reaction?
 - (c) Are accurate dilutions possible, and if so, by what technic?
 - (d) Are agglutinins resistant to deleterious influences?
 - (e) Compare the value of this method with the one used in the preceding experiment.

EXPERIMENT 56.—MACROSCOPIC AGGLUTINATION REACTION:

1. Secure serum from a rabbit which has been immunized with *Bacillus typhosus*. Serum of a typhoid convalescent may be used instead.
2. Prepare a series of serum dilutions in small narrow test-tubes in amounts of 1 c.c., ranging from 1 : 10 up to 1 : 640.
3. Add to each 1 c.c. of the bacillary emulsion of a good twenty-four- to forty-eight-hour bouillon culture of *Bacillus typhosus* or an emulsion prepared by washing twenty-four-hour growths from agar slant cultures with normal salt solution, according to the technic given in the text. This doubles the dilutions, which now range from 1 : 20 up to 1 : 1280. Prepare the culture control.
4. Shake gently and incubate for two hours at 37° C. and then record results after tubes have been standing at room temperature for six hours. Re-examine tubes with the agglutinoscope and note the higher delicacy of such readings.
5. If a typhoid immune serum of unknown titer is used and agglutination is complete in the highest dilution, the test must be repeated with still higher dilutions in order to determine the agglutinin titer of the serum.

(a) Has agglutination occurred in the control tube? Why is this control so important in this and all agglutination reactions?

(b) Is agglutination as complete in the lower as in the higher reaction? If not, how do you explain this result? Of what practical import is this phenomenon?

(c) Describe the appearance of macroscopic agglutination.

(d) Are the agglutinated bacilli dead? To determine this, pipet off the supernatant fluids of several tubes into a germicidal solution; then add an excess of sterile normal salt solution to the sediment of agglutinated bacilli, stir up the sediment, and transfer with a sterile pipet to a sterile centrifuge tube; centrifuge thoroughly; remove the supernatant fluid with a sterile pipet and plant several loopfuls of bacteria on slants of agar and in neutral bouillon. Why is it advisable to wash the sediment?

(e) What advantages has the macroscopic over the microscopic method?

EXPERIMENT 57.—MACROSCOPIC AGGLUTINATION REACTION:

1. Prepare dilutions in amounts of 1 c.c. of a typhoid immune serum in proper test-tubes, ranging from 1 : 20 up to 1 : 1280.

2. Add one loopful of a twenty-four-hour culture of *Bacillus typhosus* to each tube, being careful to emulsify thoroughly on the side of the test-tube according to the technic given. This does not materially alter the degree of dilution. Prepare the culture control as usual.

3. Incubate and examine tubes as in the previous experiment.

What are the advantages and disadvantages of this method?

EXPERIMENT 58.—MACROSCOPIC AGGLUTINATION REACTION EMPLOYING KILLED CULTURE:

1. Prepare a formalized culture of *Bacillus typhosus* as described in the text after the Dreyer method.

2. Prepare a series of dilutions of typhoid immune serum and conduct this experiment according to the macroscopic technic, using the dead instead of a living bacillary emulsion.

(a) What are the advantages of using a killed culture?

(b) Is this method as delicate as when living cultures are used?

(c) Is spontaneous agglutination likely to occur?

(d) What constitutes a satisfactory killed culture for this reaction?

EXPERIMENT 59.—GROUP AGGLUTINATION:

1. Prepare five series of test-tubes containing 1 c.c. of dilutions ranging from 1 : 20 to 1 : 640 of a typhoid immune serum.

2. To the first series add 1 c.c. of a thirty-six-hour bouillon culture of *Bacillus typhosus*; to the second series, *Bacillus paratyphosus* "B"; to the third, *Bacillus paratyphosus* "A"; to the fourth, *Bacillus enteritidis* (Gärtner); to the fifth, *Bacillus coli*. The dilutions are thus doubled. Prepare culture controls of all five cultures, and be careful that tubes are properly labeled.

3. Incubate for one hour and record the reactions.

4. This experiment may be repeated with the serum of a typhoid convalescent patient.

(a) In which series of tubes is agglutination most complete?

(b) Discuss the question of specificity of the agglutinins.

(c) How do you explain group agglutination?

(d) Are group agglutinins present to the same degree as the main agglutinin. How may the group agglutinins be eliminated?

(e) Of what value is the agglutination reaction in showing the biologic relationship of bacteria?

(f) How would the agglutination reaction be used in the diagnosis of an unknown micro-organism?

EXPERIMENT 60.—PRO-AGGLUTINATION—AGGLUTINOIDS:

1. This very important phase of agglutination may have been noted in the previous experiments, especially if old immune sera were used, when there is a possibility that agglutinin has degenerated in agglutinoids. Agglutinoids having a stronger affinity than agglutinin for the agglutino-gen, and having lost the agglutinophore group, produce little or no agglutination and prevent the action of agglutinin until diluted out of action in the higher serum dilutions. In this way agglutination may be poor or absent in low and present in higher dilutions, an important practical fact to be remembered.

2. The action of agglutinoids may be seen by conducting a macroscopic test with an old immune serum.

3. Repeat the tests with old and fresh immune sera in dilutions of 1 : 40, 1 : 80, 1 : 60, 1 : 320, using the macroscopic and microscopic technic, as this phenomenon of pro-agglutination is not infrequently found in the routine Widal reaction in typhoid fever.

EXPERIMENT 61.—THE EFFECT OF HEAT UPON AGGLUTININS:

1. Dilute 0.1 c.c. of typhoid immune serum possessing a titer of 1 : 100 or higher, with 4.9 c.c. of saline solution (1 : 50).

2. Place 1 c.c. in each of 5 small test-tubes.

3. Heat No. 1 in a water-bath at 55° C. for one-half hour; No. 2 at 60° C. for one-half hour; No. 3 at 70° C. for one-half hour, and No. 4 at 80° C. for one-half hour. No. 5 is not heated.

4. After cooling add 1 c.c. of typhoid suspension to each tube, mix, and incubate for an hour.

5. In a series of 6 small test-tubes place 1 c.c. of 1 : 50 dilution of the antityphoid serum.

6. Place five of the tubes in a water-bath at 70° C.; remove No. 1 after ten minutes and Nos. 2, 3, 4, and 5 after fifteen, thirty, forty-five, and sixty minutes respectively.

7. After cooling add 1 c.c. of typhoid emulsion to each tube and No. 6 (unheated control).

8. In tube No. 7 place 1 c.c. of saline solution and 1 c.c. typhoid emulsion (control).

9. Place all tubes in water-bath at 38° C. for one hour and read results.

(a) Does heat influence typhoid agglutinin?

(b) Are the bacterial agglutinins equally affected by heat?

(c) Are natural agglutinins more vulnerable to heat than immune agglutinins?

(d) Are immune agglutinins easily destroyed by heat, desiccation, chemical germicides, etc.?

EXPERIMENT 62.—THE INFLUENCE OF ELECTROLYTES ON AGGLUTINATION:

1. Place 10 c.c. of formalized typhoid culture for agglutination tests and 10 c.c. of typhoid serum 1 : 50 in a centrifuge tube; incubate one hour and centrifuge very thoroughly.

2. Discard the supernatant fluid and suspend the bacilli in 5 c.c. of distilled water, breaking up the clumps as thoroughly as possible.

3. Set up the following in small test-tubes:

No. 1: suspension 1 c.c. + 1 c.c. distilled water.

No. 2: suspension 1 c.c. + 0.9 c.c. distilled water + 0.1 c.c. of 10 per cent. solution of sodium chlorid.

No. 3: suspension 1 c.c. + 0.9 c.c. distilled water + 0.1 c.c. of 0.2 per cent. solution of copper sulphate.

No. 4: suspension 1 c.c. + 0.9 c.c. distilled water + 0.1 c.c. of 0.8 per cent. solution of copper sulphate.

4. Mix each tube and incubate at 37° C. for one hour.

(a) In which tubes has agglutination taken place, and why?

(b) Discuss the relation of electrolytes to the mechanism of agglutination.

EXPERIMENT 63.—ABSORPTION OF AGGLUTININS:

1. Set up macroscopic agglutination tests with a rabbit antityphoid serum and *Bacillus typhosus*; the titer should be known beforehand so that the range of dilutions may be satisfactory.

2. Repeat, using *Bacillus paratyphosus* B.
3. Repeat, using *Bacillus coli*.
4. In 2 and 3 lower dilutions of serum will be required, the final dilutions being 1 : 10, 1 : 20, 1 : 30, 1 : 40, etc.
5. Prepare a thick suspension of typhoid bacilli by washing off three agar slants. Place 5 c.c. of saline in the first and remove the growth; pour suspension into the second, etc. Heat at 60° C. for one hour.
6. Prepare similar suspension of *Bacillus paratyphosus* B and *Bacillus coli*.
7. Place 1 c.c. of typhoid serum in each of three small centrifuge tubes. To No. 1 add 4 c.c. of the typhoid suspension; to No. 2, 4 c.c. of the paratyphoid suspension, and to No. 3, 4 c.c. of the colon suspension. Mix each tube, incubate one hour, and stand in refrigerator until next day.
8. Centrifuge each tube very thoroughly and secure the supernatant fluids which represent 1 : 5 dilutions of the absorbed serum. Discard the sediments.
9. Repeat the agglutination tests with the three micro-organisms and compare the results observed with plain serum.

(a) Does absorption of typhoid serum with *Bacillus coli* remove any of the typhoid agglutinin? Does absorption with *B. paratyphosus* A remove any, and if so, why?

(b) What are "major" and "minor" agglutinins?

(c) Did absorption with typhoid bacilli remove all of the agglutinins, and if not, why?

(d) Of what practical value is this method?

EXPERIMENT 64.—AGGLUTINATION IN VIVO:

1. Cultivate Type I pneumococci in suitable fluid medium for eighteen to twenty-four hours. Centrifuge 200 c.c. very thoroughly and suspend the cocci in 10 c.c. of saline solution, endeavoring to obtain an even suspension free of clumps.
2. Inject 5 c.c. of the suspension intravenously into a rabbit.
3. Immediately remove a little blood from the heart with a syringe and prepare smears. Remove specimens again five, ten, and fifteen minutes later and prepare smears. Stain and examine for pneumococci.
4. Inject 5 c.c. of the suspension in a second rabbit and immediately thereafter 2 c.c. of Type I antipneumococcus serum; both injections intravenously.
5. Immediately remove blood from the heart (within less than one minute after the injection of serum) and prepare films. Repeat two, three, five, ten, and fifteen minutes later. Stain and examine the films.

(a) Are there evidences of agglutination in the blood of the first rabbit? In the second?

(b) Are there any evidences of phagocytosis?

(c) Discuss the agglutinins in relation to immunity.

EXPERIMENT 65.—AGGLUTINATION FOR THE DIFFERENTIATION OF PNEUMOCOCCI:

1. Sputum should be secured from a patient with Type I or Type II pneumonia. In the absence of these, sputa may be prepared by adding 1 c.c. of Type I pneumococci to 1 c.c. of saliva and injecting a mouse intraperitoneally with 1 c.c. of the mixture. A second mouse should be injected with a similar preparation of Type II pneumococci.

2. The balance of the tests, including mouse necropsies, collecting peritoneal exudates, etc., should be conducted as described in the text.

(a) Does the mouse possess natural immunity to pneumococci?

(b) Why is the mouse employed in this test?

(c) Why is the bile control tube included?

(d) What other micro-organisms have been divided into types on the basis of immunologic reactions?

(e) Of what practical value is the agglutination test for the differentiation of pneumococci?

EXPERIMENT 66.—HEMAGGLUTININS:

1. Secure 0.1 c.c. of antihuman hemolysin prepared by giving a rabbit a series of injections of washed human erythrocytes. Inactivate the serum by heating to 55° C. for a half-hour. Dilute 1 : 40 by adding 3.9 c.c. salt solution.

2. Prepare 10 c.c. of a 1 per cent. suspension of washed human erythrocytes.

3. Into a series of six small test-tubes place 0.1, 0.2, 0.4, 0.6, 0.8, and 1 c.c. of the diluted immune serum; add 1 c.c. of suspension of blood-cells and 1 c.c. of salt solution to each tube. As a control, place 1 c.c. of corpuscle suspension and 1 c.c. of normal salt solution into a seventh tube.

4. Shake gently and place in the incubator for an hour.

5. Prepare a series of hanging-drop preparations for microscopic examination.

- (a) Describe agglutination of red corpuscles.
- (b) Are the clumps easily broken up?
- (c) What would have occurred if complement were present?
- (d) Why was it necessary to heat the fresh immune serum? Is it necessary to heat an old immune serum?

EXPERIMENT 67.—AGGLUTINATION TESTS FOR THE GROUPING OF HUMAN CORPUSCLES BEFORE BLOOD TRANSFUSION:

1. Collect in test-tubes containing 1 c.c. of citrate solution a few drops of blood from each of several persons.

2. Working with known Group II and Group III sera determine the group of which each person's corpuscles belong according to a method described in the text, employing a microscopic technic.

- (a) Into how many groups may human corpuscles be divided?
- (b) What is the difference between the Jansky and Moss classifications?
- (c) How may human sera for these tests be secured and preserved?
- (d) Of what practical importance are these tests? What lesions and symptoms may develop in agglutination and hemolysis of corpuscles *in vivo*?
- (e) How is hemolysis detected in these microscopic tests?
- (f) Discuss the influence of age in relation to the isohemagglutinins.

EXPERIMENT 68.—DIRECT AGGLUTINATION AND HEMOLYSIN TESTS PRELIMINARY TO BLOOD TRANSFUSION:

1. Secure a small amount of blood in a small dry test-tube and an additional few drops in a little citrate solution from each of 4 individuals.

2. Designate one person as a patient and the others as donors. Separate the sera by centrifuging.

3. Test the serum of the patient for agglutinins and hemolysins for the corpuscles of the donors using both a microscopic and macroscopic technic.

4. Test the serum of each donor against the corpuscles of the patient in the same manner.

- (a) What are the advantages of this direct matching of bloods?
- (b) May hemolysins be present in serum in the absence of demonstrable amounts of agglutin?
- (c) In what diseases are isohemolysins particularly likely to be found?
- (d) What are auto-agglutinins and autohemolysins?

PRECIPITINS**EXPERIMENT 69.—TITRATION OF A PRECIPITIN SERUM:**

1. Secure 1 c.c. of antihorse immune serum prepared by immunizing a rabbit with normal horse-serum.

2. Secure 1 c.c. of normal-horse serum and place 2 c.c. of the following dilutions made with normal salt solution into a series of six narrow test-tubes: 1 : 100, 1 : 500, 1 : 1000, 1 : 2000, 1 : 5000, and 1 : 10,000.

3. To each tube add 0.1 c.c. of the immune serum. The tubes must not be shaken. For exact technic consult the text.

4. Place tubes in an incubator at 37° C. and observe every thirty minutes for two hours and again after standing in a refrigerator overnight.

- (a) Describe the phenomenon of precipitation.
- (b) What are the requisites of a satisfactory precipitin serum?
- (c) What are the requisites of a satisfactory preparation of the antigen for this reaction?
- (d) Discuss the delicacy of this reaction.

EXPERIMENT 70.—TITRATION OF A PRECIPITIN SERUM:

Repeat this titration, using antihuman immune serum and normal human serum.

EXPERIMENT 71.—SPECIFICITY OF PRECIPITINS:

1. Secure 1 c.c. of each clear antihorse and antihuman serums.
2. Secure 1 c.c. each of normal horse, normal human, and normal guinea-pig serum. Prepare 1 : 100 dilutions with normal salt solution; set up the following in long narrow test-tubes:

Tube 1: 2 c.c. of normal horse serum (1 : 100) + 0.1 c.c. antihorse serum.
 Tube 2: 2 c.c. of normal horse serum (1 : 100) + 0.1 c.c. antihuman serum.
 Tube 3: 2 c.c. of normal human serum (1 : 100) + 0.1 c.c. antihuman serum.
 Tube 4: 2 c.c. of normal human serum (1 : 100) + 0.1 c.c. antihorse serum.
 Tube 5: 2 c.c. of normal guinea-pig serum (1 : 100) + 0.1 c.c. antihorse serum.
 Tube 6: 2 c.c. of normal guinea-pig serum (1 : 100) + 0.1 c.c. antihuman serum.
 Tube 7: 2 c.c. of normal salt solution + 0.1 c.c. antihorse serum (control).
 Tube 8: 2 c.c. of normal salt solution + 0.1 c.c. antihuman serum (control).

3. Do not shake the tubes; place them in the incubator at 37° C.; inspect every thirty minutes for two hours.

- (a) Discuss the question of specificity of precipitins.
- (b) Of what practical value are precipitin reactions?
- (c) Enumerate the chief points in the technic of a precipitin reaction in the differentiation of proteins.

EXPERIMENT 72.—FORENSIC BLOOD TEST:

1. Secure from the instructor two pieces of muslin or gauze containing respectively a stain of human and horse blood. These are to be numbered and the source of each stain known only to the instructor. Secure 1 c.c. each of normal human and horse serum and dilute 1 : 1000.

2. Prepare extracts of each stain as described in the text.

3. Secure 1 c.c. of clear antihuman and antihorse immune serum.

4. Set up the following in long narrow test-tubes.

Tube 1: 2 c.c. of extract No. 1 in dilution of 1 : 1000 + 0.1 c.c. of antihuman serum.
 Tube 2: 2 c.c. of extract No. 1 in dilution of 1 : 1000 + 0.1 c.c. of antihorse serum.
 Tube 3: 2 c.c. of extract No. 2 in dilution of 1 : 1000 + 0.1 c.c. of antihorse serum.
 Tube 4: 2 c.c. of extract No. 2 in dilution of 1 : 1000 + 0.1 c.c. of antihuman serum.
 Tube 5: 2 c.c. of normal human serum in dilution 1 : 1000 + 0.1 c.c. antihuman serum (control).
 Tube 6: 2 c.c. of normal horse serum in dilution of 1 : 1000 + 0.1 c.c. antihorse serum (control).
 Tube 7: 2 c.c. of normal salt solution + 0.1 c.c. of antihuman serum.
 Tube 8: 1 c.c. of normal salt solution + 0.1 c.c. of antihorse serum.

5. Do not shake tubes; place in the incubator at 37° C. and inspect every thirty minutes for two hours and after standing in the refrigerator overnight.

- (a) Inspect the controls. Are the antisera potent and satisfactory? Why is it advisable to have these controls?
- (b) Are you able to diagnose the source of each stain?

(c) In a medicolegal test, if these reactions were negative, how would you proceed further in your efforts to establish the identity of a particular stain?

EXPERIMENT 73.—BACTERIAL PRECIPITINS:

1. Inoculate two flasks each containing 50 c.c. of sterile neutral bouillon with cultures of *Bacillus typhosus* and *Bacillus coli* and cultivate at 37° C. for three weeks. Filter cultures through a sterile Berkefeld filter until clear.

2. Into a series of four small test-tubes place 2 c.c. of the following dilutions of the typhoid filtrate (precipitinogen) undiluted—1 : 2, 1 : 4, and 1 : 10. Prepare a second series of tubes with similar amounts of the same dilutions of *Bacillus coli* filtrate.

3. Add 0.1 c.c. of potent typhoid immune serum to all tubes of both series. A serum with high agglutinin titer will be satisfactory.

4. Place 2 c.c. of the undiluted typhoid and coli filtrates in separate tubes as controls. Prepare an additional control by placing 0.1 c.c. of the typhoid immune serum in 2 c.c. normal salt solution.

5. Observe the tubes at the end of a half-hour, and after one, two, and six hours.

(a) Describe a positive bacterial precipitin reaction.

(b) Has a precipitate formed with the *Bacillus coli* filtrate? With what micro-organism would typhoid immune serum be likely to produce a precipitate?

(c) Discuss the relative delicacy of precipitation and agglutination reactions in the differentiation of bacteria.

EXPERIMENT 74.—BACTERIAL PRECIPITINOGENS IN INFLAMMATORY EXUDATE:

1. Repeat the inoculation of mice with sputum containing Type I pneumococci as in Experiment 65.

2. After securing the peritoneal exudate centrifuge thoroughly and conduct precipitin tests with Type I antipneumococcus serum as described in the text.

3. If urine from a case of pneumonia due to Type I pneumococci is available, repeat the tests as described in the text.

(a) What is the source of the precipitinogens in exudate and urine?

(b) Discuss the practical applications.

EXPERIMENT 75.—THERMOPRECIPITINOGENS:

1. Centrifuge 50 c.c. of a broth culture of Type I pneumococci. Suspend the cocci in 4.5 c.c. distilled water and add 0.5 c.c. of antiformin. Boil for several minutes or until the cocci are dissolved as shown by translucency. Add several drops of 1 per cent. alcoholic solution of phenolphthalein and then just enough N/1 hydrochloric acid to discharge the color. Add 15 c.c. of 95 per cent. alcohol, mix, and centrifuge a half-hour later. Add about 2 c.c. of saline solution to the sediment and boil for five minutes. Centrifuge and use the supernatant fluid (precipitinogen).

2. In a small test-tube place 0.5 c.c. of Type I antipneumococcus serum and carefully overlay with 0.5 c.c. of the precipitinogen. Observe for the formation of a ring at the line of contact.

(a) Discuss the influence of heat upon precipitinogens in general with special reference to precipitin tests for anthrax, pneumonia, and the differentiation of meats.

EXPERIMENT 76.—NOGUCHI GLOBULIN REACTION:

1. Secure 1 c.c. each of several cerebrospinal fluids from cases of paresis, tuberculous meningitis, serous meningitis, and normal human subjects.

2. Conduct this floccule-forming reaction after the technic given in the text.

3. Make total cell counts on each fluid.

(a) Explain the appearance of a positive reaction.

(b) What constitutes a negative reaction?

- (c) What is the diagnostic value of this reaction in syphilis?
- (d) What other value has this reaction in diagnosis?
- (e) What relation does this reaction bear to total cell counts?
- (f) Explain the mechanism of the reaction.

EXPERIMENT 77.—FLOCCULATION REACTIONS IN SYPHILIS:

1. Secure 1 c.c. of a cholesterolized alcoholic extract of beef heart and quickly add 3 c.c. of saline solution. Invert the mixture several times to secure an opalescent emulsion.
2. Secure sera from several syphilitic individuals yielding strongly positive Wassermann reactions; also serum from a healthy non-syphilitic individual.
3. Place 0.3 c.c. of each serum in small test-tubes and add 0.05 c.c. of the diluted extract.
4. Mix the contents of each tube gently and incubate at 38° C. for sixteen to eighteen hours, making a preliminary inspection at the end of one hour for evidences of flocculation (see Chapter XXIV).

- (a) Have flocculi developed in any of the tubes?
- (b) Discuss the probable mechanism of flocculation in syphilis.

AMBOCEPTORS AND COMPLEMENTS—HEMOLYSINS AND HEMOLYSIS

EXPERIMENT 78.—RESISTANCE OF RED BLOOD-CORPUSCLES TO SALT SOLUTION OF VARIOUS TONICITIES. NON-SPECIFIC HEMOLYSIS:

1. Arrange a series of dilutions of sodium chlorid in distilled water ranging from 0.7 to 0.2 per cent. as described in Chapter XX.
 2. Secure human blood as described and conduct the tonicity tests.
 3. Repeat at the same time, using defibrinated sheep blood.
- (a) What is the appearance of hemolyzed blood?
 - (b) What is the meaning of the term *hemolysis*?
 - (c) Why is this called *non-specific hemolysis*?
 - (d) What does a *normal* or *physiologic salt solution* mean?
 - (e) What is the importance of using a normal salt solution in hemolytic experiments?
 - (f) How is hemolysis produced by hypotonic solutions?
 - (g) What would be the objections of using a hypertonic salt solution?
 - (h) How is an isotonic salt solution prepared?
 - (i) What is the meaning of the terms *minimal and maximal resistance of red blood-corpuscles*?
 - (j) Of what practical value is this test?
 - (k) What are the average points of minimal and maximal resistance of red blood-corpuscles of healthy human beings.
 - (l) What influence has defibrination on these values?

EXPERIMENT 79.—SAPONIN HEMOLYSIS:

1. Prepare a 1 : 1000 solution of Merck's saponin in physiologic saline solution.
 2. Collect human blood and conduct the tests as described in Chapter XX using unwashed and washed corpuscles. Employ 16 dilutions of saponin varying from 1 : 20,000, 1 : 24,000, 1 : 24,000, etc., to 1 : 50,000.
 3. Repeat, using defibrinated sheep blood.
 4. Repeat, using citrated guinea-pig blood.
- (a) Is saponin hemolysis specific or non-specific?
 - (b) What is the mechanism of saponin hemolysis?
 - (c) Does the presence of serum protect the corpuscles against saponin?

EXPERIMENT 80.—BACTERIAL HEMOLYSIS:

1. Secure an eighteen- to twenty-four-hour broth culture of *Staphylococcus aureus*; also one of a hemolytic streptococcus.

2. Place 0.1, 0.2, 0.4, 0.6, 0.8, 1, and 2 c.c. of each culture in two sets of small test-tubes.
 3. Arrange a third set of tubes carrying similar amounts of sterile broth.
 4. To each tube add 1 c.c. of a 1 per cent. suspension of washed sheep corpuscles and sufficient saline solution to make the total volume 3 c.c. in each tube.
 5. Put up a control with 1 c.c. of corpuscle suspension and 2 c.c. of saline solution.
 6. Mix well and incubate in a water-bath at 38° C. for one hour.
 7. Read the results; then place tubes in a refrigerator overnight and record the results.
- (a) Has hemolysis been produced?
 - (b) Are the bacterial hemolysins specific or non-specific?
 - (c) May the culture-medium itself be hemolytic, and if so, what constituents may produce hemolysis?

EXPERIMENT 81.—THE HEMOLYTIC ACTIVITY OF ACIDS AND ALKALIES:

The object of this experiment is to demonstrate the hemolytic activity of acids and alkalies as bearing upon the question of *clean* glassware in hemolytic work, and particularly complement-fixation tests.

1. Into a series of six test-tubes place increasing amounts of a solution of hydrochloric acid prepared by diluting 1 c.c. of the normal solution with 99 c.c. of normal salt solution: 0.2, 0.4, 0.6, 0.8, 1, and 2 c.c. respectively.
 2. Into a second series of six test-tubes place increasing amounts of a solution of sodium hydroxide prepared by diluting 1 c.c. of the normal solution with 99 c.c. of normal salt solution: 0.2, 0.4, 0.6, 0.8, 1, and 2 c.c. respectively.
 3. Make the total volume in each tube equal 2 c.c. with the addition of normal salt solution.
 4. To each tube and a normal salt solution control add 1 c.c. of a 2½ per cent. suspension of washed sheep cells. Mix and stand aside for an hour or two.
- (a) Has hemolysis occurred in any of the tubes?
 - (b) What are the smallest amounts of acid and alkali producing complete hemolysis in this experiment?
 - (c) Of what practical significance are these results?
 - (d) How should test-tubes be prepared for hemolytic work?

EXPERIMENT 82.—SERUM HEMOLYSIS IN VITRO:

1. Secure 2 c.c. of blood from the ear of a rabbit which has received at least two intravenous injections of sheep cells. Separate the serum and divide into two portions. Inactivate one portion (A) by heating in a water-bath for a half-hour at 56° C.
 2. Place 0.2 c.c. of the fresh unheated serum of portion (B) in a test-tube. Likewise the same amount of the heated serum (A) in a tube. Add 1 c.c. of a 1 per cent. suspension of washed sheep cells to each tube and sufficient salt solution to bring the total volume to 3 c.c. As a control, place 1 c.c. of corpuscle suspension and 3 c.c. salt solution in a tube. Shake gently, and incubate for an hour in a water-bath at 38° C.
- (a) Which tube shows hemolysis?
 - (b) What substances are concerned in serum hemolysis?
 - (c) Why did hemolysis occur with the unheated and not with the heated serum? What is the meaning of *inactivation of a serum*?
 - (d) Why is this called serum hemolysis?
 - (e) What is the appearance of the control tube? Why is this control included? What would have happened if a hypotonic salt solution had been used?

EXPERIMENT 83.—SERUM HEMOLYSIS IN VIVO:

1. Immunize a rabbit with three intravenous injections of 5 c.c. each of a 10 per cent. suspension of washed cat erythrocytes in sterile salt solution. Give the injections each day; four days after the last injection the rabbit serum is titrated and usually contains a fair amount of anticat hemolysin.
2. Heat 5 c.c. of the immune serum at 56° C. for thirty minutes and inject into the external jugular vein of a cat.

3. Place the animal in a metabolic cage and collect the urine.

4. After two or three days, autopsy, removing the spleen, liver, and kidneys. Place in 5 per cent. formalin and prepare and stain sections with hematoxylin and eosin and Giemsa solution.

(a) Has blood destruction occurred? What are the evidences?

(b) Would hemolysis occur in the test-tube with a heated immune serum? If not, why not?

(c) How then do you explain hemolysis *in vivo* with a heated serum?

(d) Examine sections of the spleen, liver, and kidneys. Are there any evidences of phagocytosis of blood-cells, focal necrosis, and nephritis? Explain the probable mechanism of the production of these changes.

EXPERIMENT 84.—TITRATION OF ANTISHEEP HEMOLYSIN:

1. Secure 1 or 2 c.c. of blood from the ear of a rabbit which has been immunized with injections of sheep cells. Separate the serum and inactivate by heating to 56° C. for a half-hour. Dilute 1 : 100 by adding 0.1 c.c. serum to 9.9 c.c. physiologic saline solution.

2. Prepare 40 c.c. of a 5 per cent. dilution of fresh guinea-pig serum to be used for complement. Prepare 40 c.c. of a 2½ per cent. suspension of washed sheep cells by adding 1 c.c. of corpuscles to 39 c.c. of physiologic saline solution.

3. Proceed with the titration as given in the text on page 408. If the smallest dose, *viz.*, 0.1 c.c. of the 1 : 100 dilution, completely hemolyzes the corpuscles, it will be necessary to retitrate with a dilution of 1 : 1000.

(a) Is it necessary to use exactly the same amounts of complement and corpuscles in all tubes, and if so, why?

(b) If hemolysis did not occur at all in this experiment, what factors may be at fault?

(c) If the corpuscle control were completely hemolyzed, what deduction would you draw?

(d) What is the hemolytic *unit* of this serum?

EXPERIMENT 85.—QUANTITATIVE FACTORS IN SERUM HEMOLYSIS:

1. Having determined the *hemolytic unit* of the above antisheep immune serum, proceed as follows:

2. To a series of four test-tubes add an amount of immune serum equaling one, two, three, and five hemolysin units respectively. To each tube add 0.5 c.c. of the 1 : 20 dilution of complement serum. This amount is just half the dose of complement used in titrating the hemolysin. Add 1 c.c. of a 2½ per cent. suspension of sheep cells to each tube and sufficient salt solution.

3. In a second series of four test-tubes place one-half an amboceptor unit and the following amounts of diluted complement serum: 1, 2, 3, and 4 c.c. Add 1 c.c. of the suspension of sheep corpuscles and sufficient salt solution to make the total volume in each tube about equal. Incubate for one hour and read the results.

4. In a third series of four test-tubes place one amboceptor unit, 1 c.c. of complement serum (1 : 20), and the following amounts of corpuscle suspension: 1, 2, 3, and 4 c.c. Add sufficient salt solution to make the total volume in each tube about equal.

5. Prepare a corpuscle control with 1 c.c. of suspension and 4 c.c. normal salt solution.

6. Shake all tubes gently and incubate for two hours at 37° C.

(a) Can an excess of hemolysin make up for a deficiency in complement?

(b) Can an excess of complement make up for a deficiency in hemolysin?

(c) What happens when an excess of corpuscle suspension is used?

(d) Discuss the importance of quantitative factors in serum hemolysis.

EXPERIMENT 86.—RÔLE OF HEMOLYSIN AND COMPLEMENT IN HEMOLYSIS:

1. Prepare a 2½ per cent. suspension of washed sheep corpuscles. Bleed a healthy guinea-pig under ether anesthesia, separate the serum, and dilute 1 : 20 with normal salt solution (complement). Secure antisheep hemolytic serum (inactivated) whose hemolytic titer is known (consult instructor).

2. Proceed to set up a series of four test-tubes as follows:

Tube 1: 1 c.c. corpuscle suspension + sufficient normal salt solution to make the total volume about 3 c.c.

Tube 2: 1 c.c. corpuscle suspension + 1 c.c. complement serum (1 : 20) + sufficient salt solution.

Tube 3: 1 c.c. corpuscle suspension + two hemolytic units of antisheep hemolysin + salt solution.

Tube 4: 1 c.c. corpuscle suspension + 1 c.c. complement serum + two units of hemolysin + salt solution.

3. Shake each tube gently and incubate at 37° C. for one or two hours.

- (a) In which tube has hemolysis occurred? Explain the results.
- (b) What does inactivation of a serum mean?
- (c) Could some hemolysis occur, using the complement serum and corpuscles without immune hemolysin?
- (d) Could hemolysis occur in the absence of complement?

EXPERIMENT 87.—SPECIFICITY OF HEMOLYSINS:

1. Prepare a 2½ per cent. suspension of washed sheep corpuscles and a 1 per cent. suspension of washed human corpuscles. Bleed a guinea-pig under ether, separate serum, and dilute 1 : 10 with normal salt solution. Secure antisheep and antihuman hemolytic serums (inactivated) whose hemolytic titers are known.

2. Proceed as follows:

Tube 1: 1 c.c. sheep corpuscles + 1 c.c. complement (1 : 10) + two units of anti-sheep hemolysin + salt solution up to 3 c.c.

Tube 2: 1 c.c. sheep corpuscles + 1 c.c. complement (1 : 10) + two units of anti-human hemolysin.

Tube 3: 1 c.c. human corpuscles + 1 c.c. complement (1 : 10) + two units of anti-human hemolysin.

Tube 4: 1 c.c. human corpuscles + 1 c.c. complement (1 : 10) + two units of anti-sheep hemolysin.

Tube 5: 1 c.c. sheep corpuscles + 2 c.c. salt solution (control).

Tube 6: 1 c.c. human corpuscles + 1 c.c. salt solution (control).

3. Shake tubes gently and incubate for an hour or two.

- (a) In which tubes has hemolysis occurred?
- (b) What does this experiment teach as to the specificity of these amboceptors?
- (c) Discuss the specificity of hemolytic and bacteriolytic amboceptors.
- (d) If hemolysin occurs in tube No. 2, how do you explain?

EXPERIMENT 88.—THERMOSTABILITY OF HEMOLYSINS:

1. Secure antisheep hemolysin so diluted that 1 c.c. contains one unit.

2. Prepare a 2½ per cent. suspension of sheep corpuscles.

3. Secure fresh guinea-pig complement serum and dilute 1 : 20.

4. Place two units of hemolysin into each of six test-tubes. Place these in a water-bath and heat at 60° C.

5. Remove a tube from the water-bath after five, ten, fifteen, thirty, forty-five minutes, and one hour. After allowing them to cool, add 1 c.c. corpuscle suspension and 1 c.c. complement serum (1 : 20).

6. Place two units of hemolysin in each of four test-tubes. Heat No. 1 at 56° C. for thirty minutes; No. 2 at 60° C.; No. 3 at 70° C., and No. 4 at 80° C. After cooling add 1 c.c. of 1 : 20 complement and 1 c.c. of corpuscle suspension to each tube.

7. Set up a tube containing two units of amboceptor (not heated) + 1 c.c. corpuscle suspension + 1 c.c. complement serum (control). Shake each tube gently and incubate for one hour at 37° C.

- (a) Discuss the stability of hemolysins to heat, age, and germicides.
- (b) Discuss the relative thermostability of hemolysins and complements.

EXPERIMENT 89.—RESISTANCE OF HEMOLYSINS AND COMPLEMENT TO DESICCATION:

1. Secure 1 c.c. of antish sheep hemolytic serum and evenly distribute in an appropriately sized piece of filter-paper.
2. Repeat, using 1 c.c. of guinea-pig serum complement.
3. Permit both papers to dry at room temperature. Place both in an incubator overnight.
4. Next day or several days later, titrate the papers. Cut each into strips 5 mm. wide.
5. Place increasing lengths of the hemolysin paper in six test-tubes: 2, 5, 10, 15, 20, and 30 mm. Add 1 c.c. of 1 : 20 complement and 1 c.c. of 2½ per cent. suspension of sheep cells in each tube. Mix and incubate in a water-bath for one hour.
6. Arrange a second series of tubes carrying similar amounts of complement paper. To each tube add the smallest hemolytic unit of hemolysin in paper. (Note: If the 2 mm. length produces complete hemolysis, it may be used as the dose of hemolysin.) Add 1 c.c. of 2½ per cent. corpuscle suspension and 1 c.c. of saline solution to each tube. Mix and place in a water-bath for one hour.

Discuss the relative stability of hemolysins and complement to desiccation.

EXPERIMENT 90.—MECHANISM OF HEMOLYSIN ACTION:

1. Secure antish sheep hemolysin so diluted that 1 c.c. contains one hemolytic unit.
 2. Prepare a 2½ per cent. suspension of sheep corpuscles.
 3. Secure fresh guinea-pig serum and dilute 1 : 20.
 4. Into two centrifuge tubes place two units of hemolysin and 1 c.c. of corpuscle suspension. Mix and place one tube in a glass of cracked ice, leaving the other at room temperature. After one hour centrifuge both. Pipet the supernatant fluids into two separate test-tubes.
 5. To the sedimented corpuscles in both centrifuge tubes add 1 c.c. diluted complement serum and 2 c.c. of saline solution. Mix well. Incubate tubes for one hour at 37° C.
 6. To the supernatant fluid of each tube add 1 c.c. corpuscle suspension and 1 c.c. of diluted complement serum. Mix. Incubate tubes for an hour.
- (a) In which tubes has hemolysis occurred?
 - (b) How do you explain the results?
 - (c) Discuss the prevailing views of hemolysin action.

EXPERIMENT 91.—A FURTHER STUDY OF THE MECHANISM OF HEMOLYSIN ACTIVITY:

1. In a centrifuge tube place two units of antish sheep hemolysin and 2 c.c. of fresh guinea-pig complement diluted 1 : 20. Place in a glass of cracked ice until the mixture is thoroughly chilled. Then add 2 c.c. of a 2½ per cent. suspension of sheep cells (also chilled). Mix and keep at a low temperature for an hour, centrifuge rapidly, and pipet the supernatant fluid to a separate test-tube.
 2. In a second centrifuge tube place 2 c.c. of diluted complement and 2 c.c. corpuscle suspension. Keep at room temperature for one hour, centrifuge, and pipet the supernatant fluid into a test-tube.
 3. Proceed as follows: To 2 c.c. of the supernatant fluid from the first centrifuge tube add 1 c.c. corpuscle suspension; to the remaining 2 c.c. add 1 c.c. corpuscle suspension and two units of hemolysin; to the sedimented corpuscles add 2 c.c. of diluted complement serum.
 4. To 2 c.c. of the supernatant fluid from the second centrifuge tube add 1 c.c. corpuscle suspension; to the remaining 2 c.c. add 1 c.c. corpuscle suspension and two units of hemolysin. To the sedimented corpuscles add 1 c.c. of complement and 1 c.c. of saline solution.
 5. Shake all tubes gently and incubate for one hour at 37° C.
- (a) In which tubes has hemolysis occurred?
 - (b) Does complement unite directly with corpuscles?
 - (c) What evidence have you that hemolysin unites directly with corpuscles?
 - (d) What is meant by the term "sensitizing corpuscles"?
 - (e) Does hemolysis occur at a low temperature?
 - (f) What temperature best favors hemolysis?

EXPERIMENT 92.—NATURAL HEMOLYSINS:

1. Place 0.2 c.c. of serum from each of 4 normal rabbits in four small test-tubes. To each add 1 c.c. of 1 per cent. sheep corpuscle suspension.
 2. Secure six fresh human sera.
 3. Place 0.2 c.c. of each in six test-tubes and add 1 c.c. of 1 per cent. sheep corpuscle suspension to each.
 4. Repeat, using a suspension of washed guinea-pig corpuscles.
 5. Incubate the three sets of tubes in a water-bath for one hour and read the results.
 - (a) What are natural or normal hemolysins?
 - (b) Are they specific?
 - (c) What natural hemolysins may be formed in human sera?
 - (d) What influence may natural hemolysins have on complement-fixation tests?
 - (e) Are natural hemolysins for human corpuscles present in human sera?
- What are they called?
- (f) Are there any evidences of hemagglutination?

EXPERIMENT 93.—RESISTANCE OF NATURAL HEMOLYSINS TO HEAT:

1. Secure 1 c.c. of each of six fresh human sera employed in Experiment 92 and place 0.2 c.c. of each in six small test-tubes respectively. Place these in a water-bath at 55° C. for one-half hour.
2. Place 0.2 c.c. of each serum in a second set of small tubes and heat in the same manner.
3. To each tube of the first set add 1 c.c. of 1 : 20 complement and 1 c.c. of 2½ per cent. sheep corpuscle suspension.
4. To each tube of the second set add 1 c.c. of 1 : 20 complement and 1 c.c. of 2½ per cent. guinea-pig corpuscle suspension.
5. Mix well and place in a water-bath at 38° C. for one hour.

Discuss the resistance of different natural hemolysins to heat.

EXPERIMENT 94.—REMOVAL OF NATURAL HEMOLYSINS FROM HUMAN SERA:

1. Secure 1 c.c. of each of four human sera known to contain natural antish sheep hemolysin.
2. Heat each serum at 55° C. for fifteen minutes.
3. Place 0.2 c.c. of each in test-tubes and add 1 c.c. of 1 : 20 complement and 1 c.c. of 2½ per cent. sheep corpuscle suspension. Incubate one hour.
4. To the balance of each serum add 2 drops of washed undiluted sheep corpuscles after the last centrifuging; mix well and centrifuge after standing thirty minutes.
5. Place 0.2 of each supernatant serum in small test-tubes and add 1 c.c. of 1 : 20 complement and 1 c.c. of sheep-corpuscle suspension to each. Mix and incubate for one hour.
 - (a) What are the evidences of removal of hemolysin?
 - (b) Why were the sera heated?
 - (c) How would you remove hemolysin from an active or unheated serum?

EXPERIMENT 95.—RAPIDITY OF ABSORPTION OF HEMOLYSIN BY CORPUSCLES:

1. In each of a series of four small test-tubes or centrifuge tubes place 5 units of anti-sheep hemolysin (so diluted that the unit is 0.2 c.c.).
2. To each tube add 1 c.c. of 2½ per cent. sheep-cell suspension and mix well.
3. Immediately and rapidly centrifuge tube No. 1; centrifuge No. 2 at the end of five minutes, No. 3 after ten minutes, and No. 4 after fifteen minutes.
4. Place the supernatant fluids in test-tubes and add 1 c.c. of 1 : 20 complement and 1 c.c. of a 2½ per cent. sheep corpuscle suspension to each.
5. Prepare a control carrying 2 units of hemolysin, 1 c.c. of 1 : 20 complement, and 1 c.c. of corpuscle suspension. Also a second control carrying 1 c.c. of corpuscle suspension and 2 c.c. of saline solution.
6. Mix contents of each tube and place in a water-bath at 38° C. for one hour.

(a) Discuss the rapidity of absorption of hemolysin by homologous corpuscles.

(b) Would antishoop hemolysin be absorbed by human corpuscles?

(c) Discuss the influence of temperature upon the rate of hemolysin absorption.

EXPERIMENT 96.—AMOUNT OF HEMOLYSIN ABSORBED BY CORPUSCLES:

1. Titrate antishoop hemolysin with 1 c.c. of 1 : 20 complement, 1 c.c. of 2½ per cent. sheep cells, 1 c.c. of saline solution, and water-bath incubation for one hour.

2. After the unit has been determined (and the hemolysin should be so diluted that the unit is not more than 0.2 c.c.) place 2, 4, 6, 8, 10, 15, and 20 units in six centrifuge tubes respectively.

3. To each tube add 1 c.c. of 2½ per cent. sheep cell suspension and mix well.

4. After standing one-half hour in the incubator, centrifuge each tube and pipet the supernatant fluids to test tubes. Discard the sediments of corpuscles.

5. To each tube add 1 c.c. of 1 : 20 complement and 1 c.c. of 2½ per cent. suspension of sheep cells. Mix and place in a water-bath for one hour.

(a) How much hemolysin was absorbed?

(b) Discuss the mechanism of hemolysin absorption.

EXPERIMENT 97.—DISSOCIATION OF HEMOLYSIN:

1. In a test-tube place 1 c.c. of 5 per cent. sheep corpuscles and 20 units of hemolysin; mix and place in a water-bath for thirty minutes.

2. Centrifuge and set aside the supernatant fluid. This is Tube A.

3. Wash the cells three times, using 3 c.c. of saline solution for each washing, and set aside the supernatant fluid of the last washing. This is Tube B.

4. To the washed corpuscles add 3 c.c. of saline solution; mix well and place in water-bath for one hour. Centrifuge and set aside supernatant fluid, which is Tube C.

5. To the corpuscles add 1 c.c. of 1 : 10 complement and 4 c.c. of salt solution; Tube D.

6. To Tubes A, B, and C add 1 c.c. of 5 per cent. corpuscles and 1 c.c. of 1 : 10 complement.

7. Place all tubes in water-bath for one hour.

Are there any evidences of dissociation of hemolysin?

EXPERIMENT 98.—HEMOLYTIC COMPLEMENT:

1. After giving a rabbit three intravenous injections of 5 c.c. of a 10 per cent. suspension of washed sheep cells at intervals of three days, remove 3 c.c. of blood from an ear and separate the serum. Dilute the serum 1 : 10 with salt solution.

2. Into four test-tubes place 1 c.c. of a 2½ per cent. suspension of sheep corpuscles and increasing amounts of the above dilution of rabbit serum (must be fresh—not over twelve hours old) as follows: 0.4, 0.8, 1, and 2 c.c.; add sufficient salt solution. Shake and incubate for one hour.

(a) Has hemolysis occurred?

(b) How do you explain the reaction?

(c) From where was the complement derived?

(d) Are complements found in the bloods of all animals?

(e) Discuss the question of the multiplicity of complements.

EXPERIMENT 99.—INACTIVATION AND REACTIVATION OF COMPLEMENT:

1. Heat 1 c.c. of the 1 : 10 dilution of fresh rabbit immune serum used in the preceding experiment to 56° C. for a half hour (in a water-bath).

2. In a test-tube place 1 c.c. of this heated serum and 1 c.c. of a 2½ per cent. suspension of sheep cells and sufficient salt solution. Shake gently and incubate for one hour.

(a) Does hemolysis occur?

(b) How do you explain the result?

(c) What is meant by inactivation of complement?

(d) Is complement thermostable?

3. Add to the tube 1 c.c. of fresh guinea-pig serum diluted 1 : 10 and reincubate for one hour.

- (a) Has hemolysis occurred?
- (b) How do you explain the result?
- (c) What is meant by reactivation?

EXPERIMENT 100.—THERMOLABILITY OF COMPLEMENT:

1. Secure antishoop amboceptor the hemolytic unit of which is known by previous titration.
2. Prepare a $2\frac{1}{2}$ per cent. suspension of washed sheep corpuscles.
3. Into a series of four test-tubes place the following:

Tube 1: 1 c.c. of fresh guinea-pig serum diluted 1 : 20.

Tube 2: 1 c.c. of guinea-pig serum (1 : 20) which has been kept at room temperature for two days.

Tube 3: 1 c.c. of guinea-pig serum (1 : 20) three days old.

Tube 4: 1 c.c. of guinea-pig serum (1 : 20) five days old.

Add 1 c.c. of a $2\frac{1}{2}$ per cent. suspension of sheep corpuscles, two hemolytic units of antishoop amboceptor, and sufficient normal salt solution to each tube. Shake gently and incubate at 37° C. for one hour.

4. Into a series of six test-tubes place 1 c.c. of fresh guinea-pig complement serum diluted 1 : 20. Place these in a water-bath at 56° C. At intervals of ten, twenty, thirty, forty, fifty, and sixty minutes remove a tube, add 1 c.c. corpuscle suspension, two units of amboceptor, and sufficient salt solution. Shake gently each tube and incubate for one hour at 37° C.

(a) Record the results. Does hemolytic complement deteriorate readily at room temperature?

(b) What are complementoids?

(c) What practical significance has this experiment? Should a complement serum be fresh when used in hemolytic work?

(d) Is complement thermolabile? How long does it take to destroy a diluted complement at 56° C.?

(e) In inactivating a serum why do we not use a higher temperature?

EXPERIMENT 101.—EFFECT OF ACIDS, ALKALIES, AND FILTRATION UPON COMPLEMENT:

1. In each of three small test-tubes place 1 c.c. of 1 : 20 complement serum.

2. To No. 1 add 1 c.c. of 1 : 300 dilution in saline solution of N/1 solution of sodium hydroxid; to No. 2 add 1 c.c. of a 1 : 300 dilution of N/1 solution of hydrochloric acid. To No. 3 add 1 c.c. of saline solution. Mix and place in a water-bath for thirty minutes. Then add 2 units of antishoop hemolysin and 1 c.c. of $2\frac{1}{2}$ per cent. sheep corpuscle suspension to each tube; mix and reincubate for one hour.

3. Filter 5 c.c. of 1 : 20 complement through a small Kitasato or other earthen filter. Place 1 c.c. of the filtrate in a test-tube; add 2 units of hemolysin and 1 c.c. of corpuscle suspension. Mix and incubate one hour.

4. Set up a corpuscle control.

(a) Does sodium hydroxid and hydrochloric acid destroy complement?

(b) What would be the effect of using larger amounts?

(c) What relation do these results bear to the preparation of glassware for complement fixation tests?

(d) Is complement-filtrable?

EXPERIMENT 102.—TITRATION OF HEMOLYTIC COMPLEMENT:

1. Prepare 20 c.c. of complement 1 : 20; prepare a $2\frac{1}{2}$ per cent. suspension of sheep cells,

2. To a series of 10 test-tubes add increasing doses of diluted complement serum: 0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, and 1.5 c.c.; add 2 units of hemolysin (determined by previous titration), 1 c.c. of corpuscle suspension, and sufficient salt solution to bring the total volume in each tube to 3 c.c.

3. Shake gently and incubate for one hour at 37° C.

- (a) Record the results. What is the complement unit of this serum?
- (b) Is the complement content of the serums of different animals constant? Does it vary in animals of the same species? In the same animal at different times?

EXPERIMENT 103.—COMPLEMENT ACTIVITY OF DIFFERENT ANIMAL SERA:

1. Secure 0.5 c.c. of fresh human, guinea-pig, and rabbit serum. Dilute each with 4.5 c.c. of saline solution (1 : 10).
2. Test each serum for complement and natural antisheep hemolysin by placing 0.5 c.c. in test-tubes with 1 c.c. of 2½ per cent. sheep-cell suspension. Mix and incubate one hour.
3. Arrange three series of six test-tubes each. Into the first set place 0.1, 0.2, 0.4, 0.6, 0.8, and 1 c.c. of the diluted human serum. Place similar amounts of the guinea-pig serum in the second set and rabbit serum in the third set.
4. To each tube add 2 units of antisheep hemolysin (based upon titration with 1 c.c. of 1 : 20 guinea-pig complement and 1 c.c. of 2½ per cent. sheep corpuscle suspension) and 1 c.c. of 2½ per cent. sheep corpuscle suspension.
5. Set up a corpuscle control.
6. Mix contents of each tube and place in a water-bath for one hour.

- (a) Are the different sera of equal hemolytic activity?
- (b) Are the differences due to variation in amounts of complement or natural antisheep hemolysin, or both?
- (c) What animal serum is best adapted for complement in complement-fixation tests?

EXPERIMENT 104.—PRESERVATION OF COMPLEMENT:

1. Dissolve 2 grams of chemically pure sodium chlorid in 10 c.c. of fresh guinea-pig serum. Keep in a refrigerator.
2. Immediately after the solution has been prepared, dilute 1 c.c. with 19 c.c. of distilled water, which gives 20 c.c. of a 1 : 20 dilution of complement in isotonic solution.
3. Titrate the diluted complement with 2 units of hemolysin, 1 c.c. of 2½ per cent. sheep-cell suspension, and sufficient saline to make 3 c.c. Water-bath incubation one hour.
4. Repeat the titration three days later, again at the end of a week, and thereafter at weekly intervals. Wassermann tests may also be performed.

- (a) Discuss the influence of temperature upon the preservation of complement serum.
- (b) What other methods may be employed?
- (c) How long may complement serum be preserved?
- (d) What are the signs of deterioration?
- (e) Does hemolytic activity deteriorate sooner or later than fixability by syphilis antigen and antibody?
- (f) What are the practical advantages of using preserved complement?

EXPERIMENT 105.—PHENOMENON OF COMPLEMENT FIXATION:

This experiment is introduced here to show the specific Bordet-Gengou phenomenon of complement fixation. The exact technic of complement-fixation reactions as conducted for the diagnosis of syphilis and other infections will be given in subsequent exercises.

1. Dilute 1 c.c. of guinea-pig serum complement with 9 c.c. of saline solution (1 : 10) and titrate in doses of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 c.c. against 2 units of hemolysin and 1 c.c. of 2½ per cent. sheep corpuscle suspension.
2. Secure 1 c.c. of an antigenococcus and a normal serum and heat each at 56° C. for thirty minutes.
3. Secure an emulsion of gonococci which is now called the *antigen*; for the proper dose to use consult the instructor, or a typhoid immune serum and typhoid antigen may be employed.

4. Proceed as follows:

- Tube 1: 0.2 c.c. antigonococcus serum + dose of antigen + 2 units of complement + normal salt solution.
- Tube 2: 0.2 c.c. antigonococcus serum + 2 units of complement + normal salt solution (serum control).
- Tube 3: 0.2 c.c. normal serum + dose of antigen + 2 units of complement + normal salt solution.
- Tube 4: 0.2 c.c. normal serum + 2 units of complement + normal salt solution (serum control).
- Tube 5: dose of antigen + 2 units of complement + normal salt solution (antigen control).
- Tube 6: 2 units of complement + normal salt solution (hemolytic control).
- Tube 7: 1 c.c. corpuscle suspension + normal salt solution (corpuscle control). Plug tube with cotton as it is finished.

5. Shake all tubes gently and incubate for one hour at 37° C.

6. Add 2 units of hemolysin and 1 c.c. corpuscle suspension to all tubes except corpuscle control. Shake gently and reincubate for one hour.

- (a) Examine tubes and record your results.
- (b) Why was the serum control on each serum necessary?
- (c) Why was the hemolytic system control necessary?
- (d) Why was the antigen control necessary?
- (e) What is meant by non-specific complement fixation?
- (f) What is meant by specific complement fixation? Explain the phenomenon. Which tube of this series shows specific complement fixation, and why? Is there any evidence of non-specific fixation in any of the tubes? If so, what bearing would this have on the results of this test?
- (g) What is meant by complement deviation? By complement fixation?
- (h) Upon what does the specificity of complement fixation depend?

ANTIGENS FOR THE WASSERMANN REACTION

EXPERIMENT 106.—PREPARATION OF ANTIGENS FOR THE WASSERMANN REACTION:

- 1. Prepare 50 c.c. of an alcoholic extract of beef heart.
- 2. Prepare an extract of acetone-insoluble lipoids from 20 grams of beef heart.
- 3. Prepare 50 c.c. of a cholesterinized alcoholic extract of human heart.
- 4. Prepare 50 c.c. of the author's new antigen.

- (a) Are antigens for the Wassermann syphilis reaction specific?
- (b) Upon what does the high specificity of the Wassermann reaction depend?
- (c) What constitutes a specific antigen for the Wassermann reaction?
- (d) Discuss the important relation of antigen to the Wassermann reaction.

EXPERIMENT 107.—METHODS OF TITRATION OF ANTIGENS:

While the above extracts are in the course of preparation, stock extracts may be used for titration. After the student has finished the above four extracts, they are titrated in a similar manner.

- 1. Dilute 1 c.c. of an alcoholic extract of beef heart in a test-tube 1 : 10 by slowly adding 9 c.c. of normal saline solution. Dilute a cholesterinized extract (1 : 20) by slowly adding 9.5 c.c. salt solution to 0.5 c.c. of extract.
- 2. Conduct the anticomplementary titration of each (page 473).
- 3. Conduct the antigenic titration of each (page 475).
- (a) What is meant by the anticomplementary dose of an antigen?
- (b) What is meant by the antigenic dose of antigen?
- (c) Why are these titrations so important?

(d) In a complement-fixation test what would be the result if the antigen were used in the anticomplementary dose? What would be the result if the antigen were used in less than the antigenic dose?

(e) What constitutes a satisfactory antigen for any complement-fixation test?

(f) In conducting a diagnostic reaction, should the antigen be used in exactly one antigenic dose or double or treble this amount? Why and under what conditions would this be a safe procedure?

(g) Why should the antigen be diluted slowly with salt solution?

(h) Why is a serum control so important in these titrations? What other controls are used, and why?

(i) Which is more important, the anticomplementary or antigenic titration, and why?

EXPERIMENT 108.—TITRATION OF ANTIGEN (CONTINUED):

Titrate the writer's new antigen for hemolytic, anticomplementary, and antigenic activities according to the new technic (page 483).

What substances in tissue extracts are largely responsible for hemolytic activity? For anticomplementary activity? For antigenic activity?

EXPERIMENT 109.—ANTICOMPLEMENTARY ACTION OF SERA:

1. Secure 1 c.c. of five different specimens of human sera which have been standing in the laboratory for one, two, four, six, and ten days respectively. The last specimen should be intentionally contaminated with a culture of staphylococcus.

2. Divide each serum into two portions and heat portion "B" to 56° C. for a half-hour.

3. Place 0.2 c.c. of each specimen (unheated) in a series of five test-tubes.

4. Place 0.2 c.c. of each specimen (heated) in a second series of five test-tubes.

5. To each tube add 1 c.c. of complement 1 : 20 and sufficient salt solution to bring the total volume to 3 c.c. Shake gently and incubate for a half-hour. Add 2 units of hemolysin and 1 c.c. corpuscle suspension to each tube and incubate for one hour.

6. A hemolytic system control (complement, corpuscles, and amboceptor) should be included; also a corpuscle control.

(a) Record your results. Are any of the sera anticomplementary? How do you determine this?

(b) What is meant by the terms *anticomplementary action of a serum*?

(c) What significance has this phenomenon in complement-fixation work?

(d) Are anticomplementary bodies thermolabile or thermostabile? May both exist? Under what conditions are each most likely to be present?

(e) How are thermolabile anticomplementary bodies removed or their influence overcome? When a serum is three or more days old, what is the chief object of heating it before it is used in a complement-fixation test?

(f) Does complement keep for three days?

(g) Is it possible to remove the thermostabile anticomplementary action of a serum? Could such a serum be used in a complement-fixation reaction? How is this condition of the serum detected?

(h) Under what conditions is a serum likely to become anticomplementary in action? Is a sterile serum likely to become anticomplementary?

EXPERIMENT 110.—WASSERMANN REACTION (FIRST METHOD):

1. Secure four specimens of blood: one from a known syphilitic person; the second from a known normal person, the third and fourth specimens for diagnosis. Also a specimen of cerebrospinal fluid.

2. Conduct a Wassermann reaction with each serum after the *first method*, using an antigen of alcoholic extract of syphilitic liver or beef heart.

(a) Record your results. In case the hemolytic system control was not completely hemolyzed, what may be the reasons and what influence would this result have on interpreting the reactions?

(b) In case the antigen control was not completely hemolyzed, what influence would this result have on the reactions?

(c) If a serum control were incompletely hemolyzed, what influence would this have on the results of the test?

(d) Is this method a quantitative reaction?

(e) What is the nature of the antibody in a syphilitic serum?

(f) How are these reactions recorded? How should they be reported to a clinician?

EXPERIMENT 111.—AUTHOR'S MODIFICATION OF THE WASSERMANN REACTION EMPLOYING MULTIPLE ANTIGENS:

Conduct a Wassermann reaction with each of five specimens of serum after the *second method*, using an alcoholic extract of syphilitic liver or beef heart, an extract of acetone-insoluble lipoids, and an alcoholic extract of heart reinforced with cholesterolin. One of these sera should be from a syphilitic person (positive control) and one from a normal person (negative control).

(a) What are the advantages of using more than one antigen?

(b) Discuss the relative value of these three antigens.

(c) Under what conditions may a cholesterolized extract be used?

EXPERIMENT 112.—NON-SPECIFIC COMPLEMENT FIXATION:

1. Secure specimens of sera from normal rabbits and heat a portion of each at 55° C. for thirty minutes.

2. Prepare an antigen of staphylococci or typhoid bacilli by washing off twenty-four-hour agar slant cultures with saline solution; heat for one hour and titrate for anticomplementary activity. Use an amount equal to one-third the anticomplementary unit in these tests.

3. Conduct Wassermann tests after the second method, employing three tissue and the bacterial antigens.

4. Heat a fresh portion of each serum at 60° to 62° C. for thirty minutes and repeat the tests.

(a) Discuss non-specific complement fixation versus anticomplementary activity.

(b) Were any of these sera or antigens anticomplementary?

(c) What animal sera are known to give non-specific reactions?

(d) What influence does heating the sera have on the phenomenon?

EXPERIMENT 113.—NON-SPECIFIC COMPLEMENT FIXATION BY HUMAN SERA; PROTEOTROPIC REACTIONS:

1. Secure the sera of 12 healthy non-syphilitic individuals.

2. Use each serum *unheated* in dose of 0.1 c.c. and conduct Wassermann tests with three tissue antigens including a cholesterolized extract, after the Noguchi method.

3. Heat a portion of each serum at 55° C. for fifteen minutes and repeat the tests.

(a) May unheated non-syphilitic human sera yield non-specific complement-fixation reactions?

(b) What relation does that kind of antigen employed bear to these reactions?

(c) Does heating human serum increase or decrease the tendency for non-specific reactions?

EXPERIMENT 114.—THE INFLUENCE OF TEMPERATURE AND DURATION OF PRIMARY INCUBATION UPON COMPLEMENT-FIXATION REACTIONS:

1. Prepare a 2½ per cent. suspension of sheep corpuscles; also a 1 : 10 dilution of complement. Titrate the hemolysin in doses of 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 c.c., using 0.5 c.c. of corpuscle suspension, 0.3 c.c. of complement, and sufficient saline solution to make 2 c.c.

2. Now titrate the complement in dose of 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 c.c., using two units of hemolysin, 0.5 c.c. corpuscle suspension, and saline solution to make 2 c.c.

3. *Determine the influence of temperature upon complement destruction as follows:* Arrange a series of five test-tubes. Place 1, 2, 3, 4, and 5 units of complement in each respectively, with saline to make 1.5 c.c. Place in a water-bath for one hour for primary incubation. Add 2 units of hemolysin and 0.5 c.c. of corpuscle suspension and reincubate one hour. Record the unit.

Repeat, except that the primary incubation is conducted by placing the tubes in a refrigerator for eighteen hours at 6° to 10° C. Next day add 2 units hemolysin and 0.5 c.c. of the corpuscle suspension (carried over in the refrigerator) and incubate one hour. Record the unit.

4. *Determine the influence of temperature upon the anticomplementary action of antigen as follows:* Repeat the complement titrations in exactly the same manner with both kinds of primary incubation, except that 5 units of antigen are added to each tube.

5. *Determine the influence of temperature upon the anticomplementary action of serum:* Repeat the complement titrations in exactly the same manner with both kinds of primary incubation, except that 0.1 c.c. of a mixture of strongly positive syphilitic sera (heated to 55° C. for fifteen minutes) is added to each tube.

6. *Determine the influence of temperature upon the specific fixation of complement:* Arrange a series of eight test-tubes; place 0.1 c.c. of the syphilitic serum and 5 units of the antigen in each. Add increasing amounts of complement to the tubes respectively: 2, 4, 6, 8, 10, 12, 15, and 20 units. Add sufficient saline solution to equalize the tubes. Give a primary incubation of one hour in a water-bath. Add 2 units of hemolysin and 0.5 c.c. of corpuscle suspension and reincubate one hour.

Repeat, using a primary incubation of eighteen hours at 6° to 10° C.

7. Now subtract the total amount of complement destroyed, the amount fixed by antigen alone and by serum alone from that fixed by the mixture of serum and antigen in the water-bath primary incubations. The difference expresses the amount of complement fixed specifically.

8. Make the same calculations for the titrations employing the cold or refrigerator method of incubation.

(a) What influence has temperature and duration of primary incubation upon the destruction of complement? Upon the anticomplementary activity of antigen alone and serum alone? Upon the specific fixation of complement by mixtures of serum and antigen?

(b) Why may a hemolytic system and complement-fixation test satisfactory for a warm primary incubation be unsatisfactory with a prolonged cold incubation?

EXPERIMENT 115.—THE AUTHOR'S NEW COMPLEMENT-FIXATION TEST BASED UPON THE RESULTS OF STUDIES IN THE STANDARDIZATION OF TECHNIC:

Conduct tests with each of four specimens of serum after the *third method*, using the author's new antigen. One of these serums should be a positive control and one a normal or negative control.

(a) Why is a mixture of guinea-pig sera employed as complement?

(b) Why are the specimens of guinea-pig blood placed in the incubator for one hour or in a refrigerator overnight, before separation of the sera?

(c) What is the advantage of titrating the complement in the presence of the dose of antigen?

(d) What is the advantage of titrating the hemolysin each time the tests are done?

(e) Why are the doses of various reagents made 0.5 c.c. instead of employing smaller amounts?

- (f) Why are the sera heated at 55° C. for fifteen minutes?
- (g) Why are the mixtures of serum and antigen allowed to stand before the addition of complement?
- (h) What are the advantages of a primary incubation of eighteen hours at 6° to 8° C. followed by ten minutes in a water-bath at 38° C.? What is the disadvantage?
- (i) What are the advantages of a quantitative test?
- (j) What is the advantage of using a color scale?

EXPERIMENT 116.—AUTHOR'S MODIFICATION OF THE WASSERMANN REACTION EMPLOYING VARYING AMOUNTS OF COMPLEMENT:

Conduct a Wassermann reaction with a known positive, a known negative, and two unknown serums after the *fourth method*, using an extract of acetone-insoluble lipoids.

What are the main advantages of this technic?

EXPERIMENT 117.—TITRATION OF ANTIHUMAN HEMOLYSIN:

1. Secure 0.1 c.c. of inactivated antihuman rabbit hemolytic serum and dilute 1 : 40 by adding 3.9 c.c. normal saline solution.
 2. To a series of six small test-tubes add increasing amounts of diluted hemolysin as follows: 0.1, 0.2, 0.4, 0.6, 0.8, and 1 c.c., add 0.1 c.c. of 40 per cent. complement, 1 c.c. of 1 per cent. human corpuscle suspension, and sufficient saline solution to bring the total volume to 2 c.c.
 3. Incubate for one to two hours, shaking the tubes once or twice during this time.
- (a) Are there any evidences of hemagglutination?
 - (b) What constitutes the unit of hemolysin?
 - (c) Is an antihuman hemolytic system more delicate than an antish sheep system in complement-fixation work?
 - (d) What are the advantages and disadvantages of using an antihuman hemolytic system?

EXPERIMENT 118.—TECHNIC OF THE NOGUCHI MODIFICATION:

1. Secure five specimens of blood not over twenty-four hours old, including at least one positive and one negative serum.
 2. Conduct the Noguchi reaction with each serum in the unheated or *active* state, using an antigen of acetone-insoluble lipoids. (See page 496).
 3. Conduct the reactions with the same antigen, using the serums after heating to 56° C. for a half-hour.
- (a) Record your results. Are they equal in both series?
 - (b) What is meant by proteotropic reaction?
 - (c) What effect has heat upon syphilis reagin?
 - (d) Does an active serum react more delicately than an inactivated one?
 - (e) In performing this test with unheated serum what precautions are to be observed?

EXPERIMENT 119.—PREPARATION AND TITRATION OF BACTERIAL ANTIGENS:

1. Cultivate typhoid bacilli upon slants of agar for forty-eight to seventy-two hours.
2. Wash these off with sufficient saline solution to give a cloudy suspension. Shake with glass beads for one hour and heat at 60° C. for one hour. Add 0.05 c.c. of a 5 per cent. solution of phenol for each cubic centimeter of antigen.
3. Titrate the antigen for anticomplementary activity and use it in dose corresponding to one-quarter this amount.
4. Conduct complement-fixation tests with the human sera if sera from typhoid fever patients are available. If not, employ rabbit typhoid immune serum heated to 60° to 62° C. for thirty minutes. If the author's new technic is employed the maximum dose should be not more than 0.025 c.c. to avoid non-specific reactions.

- (a) Describe different methods for the preparation of bacterial antigens.
- (b) In what diseases of human beings and the lower animals are complement-fixation tests of practical diagnostic value.
- (c) Why does complement fixation fail in some bacterial diseases?
- (d) Why is complement fixation generally stronger in syphilis than in other bacterial diseases?
- (e) Why is a sensitive technic especially desirable in bacterial complement-fixation tests?

EXPERIMENT 120.—TECHNIC OF THE GONOCOCCUS REACTION:

1. Secure six specimens of serum from a genito-urinary clinic, particularly of men suffering with chronic gonococcus infections. Also a known positive and a known normal serum for controls.

2. Conduct the reactions after one of the methods described.

- (a) Is the gonococcus reaction specific?
- (b) In what cases of gonococcus infection is the reaction likely to be negative? Likely to be positive?
- (c) How soon after infection is the reaction likely to become positive?
- (d) Discuss the practical value of the gonococcus complement-fixation test.

EXPERIMENT 121.—TUBERCULOSIS COMPLEMENT-FIXATION TEST:

Secure an antigen of tubercle bacilli and conduct complement-fixation tests with the following types of sera:

- (a) From individuals with acute pulmonary tuberculosis.
- (b) From cases of chronic fibroid tuberculosis.
- (c) From healthy individuals giving negative v. Pirquet tuberculin reactions.
- (d) From syphilitic individuals giving negative v. Pirquet tuberculin reactions.

- (a) Is the tuberculosis complement-fixation test specific?
- (b) May the sera of syphilitic non-tuberculous individuals give positive reactions, and if so, why?
- (c) Discuss the practical value of the complement-fixation reaction in human tuberculosis.

EXPERIMENT 122.—COMPLEMENT FIXATION IN THE DIFFERENTIATION OF PROTEIN; TITRATION OF IMMUNE SERA:

- 1. Secure 1 c.c. each of antihuman and antihorse-serum. Inactivate both.
- 2. Secure 0.1 c.c. each of fresh human and horse-serum and dilute 1 : 1000.
- 3. Conduct an antigenic titration of each immune serum as described on page 561.

- (a) Explain the mechanism of this reaction.
- (b) Are protein amboceptors specific?
- (c) Discuss the question of inhibition of hemolysis due to a precipitin reaction.

EXPERIMENT 123.—TECHNIC OF THE FORENSIC COMPLEMENT-FIXATION BLOOD TEST:

- 1. Secure from the instructor two pieces of gauze stained respectively with human and horse blood. These are to be numbered and their identity known only to instructor.
- 2. Secure 1 c.c. each of antihuman and antihorse-serum.
- 3. Secure 0.1 c.c. of known human and horse-serum for controls.
- 4. Proceed with the test for identifying these bloods as described in the text.
- 5. Conduct precipitin tests at the same time.

- (a) Discuss the specificity of this test.
- (b) Is this test more delicate than the precipitin reaction?

(c) Which is the more liable to error in technic?

(d) Discuss the applicability of the complement-fixation technic to the differentiation of proteins in general, as animal, vegetable, and bacterial proteins.

EXPERIMENT 124.—VENOM HEMOLYSIS IN SYPHILIS:

1. Secure 1 c.c. of venom solution 1 : 1000.

2. Secure four specimens of blood (for technic see page 416) from cases of late secondary or tertiary syphilis and one specimen of normal blood. Also a known normal blood for control.

3. Prepare the subdilutions of venom and conduct the tests after the technic described on pages 416 and 417.

(a) Discuss the prevailing views regarding the mechanism of venom hemolysis.

(b) Discuss the value of the venom test in the diagnosis of syphilis.

(c) Discuss the main points in the technic.

EXPERIMENT 125.—PFEIFFER BACTERIOLYTIC TEST:

1. Secure 1 c.c. of serum from a rabbit which has been immunized with cholera bacilli. By working with cholera and a highly potent serum better results are secured, but as there is probably more danger connected with the handling of cholera cultures, typhoid may be substituted with fairly good results. Inactivate by heating to 56° C. for half an hour.

2. Prepare a twenty-four-hour-old agar culture of a virulent strain of *Bacillus cholera*.

3. Prepare a 1 : 100 dilution of the immune serum and place 3 c.c. in a small test-tube. Add three loopfuls of culture and emulsify thoroughly. Inject a young guinea-pig intraperitoneally with 2 c.c. of the emulsion.

4. At intervals of ten, twenty, forty, and sixty minutes withdraw small amounts of exudate and study bacteriolysis; prepare hanging-drop preparations which may be compared with a similar control on the culture; prepare smears of the culture and peritoneal exudate and stain with carbolthionin or carbolfuchsin.

5. If desirable, the bacteriolytic titer of the serum may be determined after the method given in the text.

(a) Describe the phenomenon of bacteriolysis.

(b) Are bacteriolysis and hemolysis similar processes? Give the source of complement in this reaction.

(c) Discuss the specificity of bacteriolytic reactions.

(d) Discuss the practical value of the bacteriolytic test in the diagnosis of cholera.

EXPERIMENT 126.—MICROSCOPIC METHOD OF MEASURING THE BACTERIOLYTIC POWER OF THE BLOOD

This method may be employed for a rapid estimation of the bacteriolysin produced in the blood in response to an inoculation of typhoid vaccine.

1. Secure a small quantity of the patient's serum by collecting blood aseptically in a Wright capsule. About 1 c.c. of serum will be sufficient. Secure a control serum, preferably a "pooled serum," in the same manner. Prepare dilutions of the patient's serum in the following manner: Place a series of six small test-tubes (sterile) in a rack; add 1 c.c. sterile broth to each tube; into the first tube place 1 c.c. of the fresh serum from the patient (1 : 2 dilution); mix well and transfer 1 c.c. to the second tube; mix and transfer 1 c.c. to the third, and so on to the last tube, when 1 c.c. is discarded.

2. Secure a twenty-four-hour culture of typhoid bacilli in neutral bouillon.

3. Take a simple capillary pipet with a long stem, plugged with cotton and sterilized, and make a mark about 3 cm. from the end. Draw up the highest dilution of serum to the pencil mark, then a bubble of air, and finally an equal volume of culture. Thoroughly mix by carefully aspirating and driving out the contents on a hollow ground slide or in a small tubule. Aspirate the mixture into the middle portion of the stem and seal the pipet in a flame. Label with the final dilution (1 : 128).

4. Proceed in the same manner with the remaining five dilutions of the patient's serum,

which then, mixed with an equal quantity of culture, equals 1 : 64, 1 : 32, 1 : 16, 1 : 8, and 1 : 4. Place these pipets in a large test-tube and label with the patient's name and time when placed in the incubator.

5. Prepare a similar set of pipets using the control serum.
6. Prepare a culture control by mixing equal volumes of culture and sterile broth.
7. Place all pipets in the incubator at 37° C. for two hours.
8. Prepare hanging-drop preparations of each pipet by breaking off the tip and placing a drop of the contents (after mixing) on a cover slide and suspending in the usual manner. First examine the culture control and then each of the various dilutions, noting in each case the dilution in which there is the first bacteriolytic effect, and the dilution in which there is a complete effect; arrive at the *bacteriolytic index* by comparing the patient's and the control blood just as the opsonic index is stated.

- (a) What are the first evidences of bacteriolysis?
- (b) Are endotoxins neutralized when bacteriolysis occurs?

EXPERIMENT 127.—METHOD OF MEASURING THE BACTERICIDAL ACTIVITY OF THE BLOOD IN VITRO (METHOD OF STERN AND KORTE):

With a culture of typhoid bacilli, rabbit typhoid immune serum, and rabbit complement serum, a test may be carried out after the method given in the text.

- (a) What main precautions are to be followed in this method?
- (b) Discuss complement deviation.
- (c) Discuss the practical value of this method.

EXPERIMENT 128.—BACTERICIDAL POWER OF NORMAL SERUM:

1. Secure 5 c.c. of blood from the vein of a person aseptically and separate the serum.
2. Place 0.5 c.c. serum in a sterile test-tube and add 0.5 c.c. of 1 : 1000 dilution of twenty-four-hour broth culture of *Bacillus typhosus*.
3. Repeat, using 1 : 5000 dilution of culture.
4. Repeat with the serum of an individual recently immunized against typhoid fever, using both dilutions of culture.
5. Place 0.5 c.c. of the typhoid serum in a sterile test-tube and heat at 56° C. for a half hour; after cooling add 0.5 c.c. of 1 : 5000 culture.
6. Place 0.5 c.c. sterile salt solution in a sterile test-tube and add 0.5 c.c. of 1 : 5000 culture (culture control).
7. Place 0.5 c.c. sterile salt solution in a sterile test-tube and add 0.5 c.c. of unheated serum (serum control).
8. Place all tubes in a water-bath at 38° C. for two hours; then pour into each a tube (about 10 c.c.) of agar which has been boiled and cooled to 42° C. Mix well and pour into sterile Petri dishes.
9. Incubate in an inverted position for twenty-four to forty-eight hours and count colonies.

- (a) What bacteriolysins may be found in normal human serum?
- (b) Does active immunization with typhoid vaccine increase typhoid bacteriolysin?
- (c) What relation may agglutinins bear to bacteriolysis?
- (d) What relation does complement bear to bacteriolysis?

EXPERIMENT 129.—BACTERICIDAL ACTIVITY OF WHOLE COAGULABLE BLOOD (HEIST-LACY METHOD):

Test the bactericidal activity of the whole blood of a chicken or pigeon for pneumococci; at the same time repeat the tests, employing human blood. The technic is described in the text.

- (a) Is natural immunity sometimes due to bactericidal substances in the blood?
- (b) Does the bactericidal activity of serum always require the presence of a complement?
- (c) What are the advantages and disadvantages of this technic?

EXPERIMENT 130.—NEISSER-WECHSBERG PHENOMENON OF COMPLEMENT DEVIATION:

1. Dilute 0.1 c.c. of a twenty-four-hour broth culture of *Bacillus cholerae* with 99.9 c.c. of sterile saline solution (1 : 1000).
2. In a series of twelve small sterile test-tubes place 1 c.c. of sterile saline solution. To No. 1 add 1 c.c. of sterile anticholera serum; mix and transfer 1 c.c. to No. 2, and so on to No. 12, from which discard 1 c.c.
3. To each tube add 0.5 c.c. of the diluted culture and 0.3 c.c. of sterile guinea-pig serum.
4. Set up Tube No. 13 with 0.5 c.c. culture and 1.3 c.c. of saline solution (culture control).
5. Set up Tube No. 14 containing 0.5 c.c. of culture, 0.3 c.c. of guinea-pig serum, and 1 c.c. of saline solution (complement control).
6. Set up Tube No. 15 containing 1 c.c. of 1 : 100 anticholera serum, 0.5 c.c. of culture, and 0.3 c.c. of saline solution.
7. Mix all tubes well and incubate three hours in a water-bath at 38° C.
8. Boil 15 tubes of agar and cool to 45° C.; arrange 15 sterile Petri dishes.
9. Place 5 drops from each tube in a tube of agar, pour into Petri dishes, and incubate twenty-four to forty-eight hours. Count the colonies.

- (a) In which tubes occurred the greatest degree of bacteriolysis?
- (b) Does the degree of bacteriolysis bear a relation to the amount of immune serum employed?
- (c) Why is bacteriolysis less marked with the larger amounts of serum?
- (d) Is normal guinea-pig serum bacteriolytic for cholera bacilli? Is normal rabbit serum?

CYTOTOXINS

EXPERIMENT 131.—ACTION OF NEPHROTOXIN:

1. Prepare an emulsion of guinea-pig kidney as described in the text and immunize a rabbit.
2. Inject a guinea-pig intravenously with 5 c.c. of rabbit immune serum per kilo of body weight.
3. Place the animal in a metabolism cage, collect all urine, and carefully examine for albumin, casts, and hemoglobin; make quantitative albumin determinations.
4. After three days autopsy and examine the kidneys and liver histologically.
5. Heat the immune serum to 56° C. for thirty minutes and place increasing amounts in a series of test-tubes as follows: 0.01, 0.05, 0.08, 0.1, and 0.2 c.c.; add 1 c.c. of fresh guinea-pig complement serum 1 : 20, and 1 c.c. of 2½ per cent. suspension of washed guinea-pig erythrocytes. Incubate for two hours.

- (a) Describe the changes occurring in the liver and kidneys. Give the prevailing views regarding the mechanism of their production.
- (b) Are there any evidences of a hemolytic action of the serum *in vivo*?
- In vitro*?
 - (c) How do you explain the presence of hemolysin in this immune serum?
 - (d) Discuss the specificity of cytotoxins in general.
 - (e) How many a hemolysin be removed from a nephrocytotoxic serum?
 - (f) Have the cytotoxins any practical value in the treatment of disease?

EXPERIMENT 132.—SPERMATOTOXIN:

1. Secure the testes of a large guinea-pig immediately after death and prepare a saline extract. Centrifuge very briefly if necessary for the removal of large particles.
2. Examine a drop under the microscope for spermatozoa, many of which should be present.
3. Inject a rabbit intraperitoneally at intervals of five days with increasing amounts, beginning with 2 c.c.
4. After five or six doses secure fresh serum from this rabbit; also from a normal rabbit.
5. Prepare a fresh suspension of guinea-pig testes containing many motile spermatozoa.
6. On cover-glasses mix two loopfuls of immune serum with one loopful of suspension of spermatozoa. Suspend in a hanging-drop slide and vaselin the edges. Repeat, using the normal serum. Repeat, using saline solution instead of serum.
7. Observe the slides at intervals over a period of several hours.

8. In a series of six small test-tubes place varying dilutions of immune serum in amounts of 1 c.c.; undiluted, 1 : 2, 1 : 4, 1 : 8, 1 : 16, and 1 : 32. Add 1 c.c. of spermatozoa suspension to each. Put a control tube using saline solution in place of serum. Mix each tube and place in a water-bath for two hours. Observe for evidences of agglutination. Examine drops microscopically for loss of motility.

Do the normal and immune sera affect the spermatozoa, and if so, in what manner?

EXPERIMENT 133.—TECHNIC OF THE MIOSTAGMIN REACTION:

1. Secure a portion of dry pulverized cancer tissue and prepare an antigen after the technic described in the text.

2. Titrate this antigen.

3. Secure four specimens of serum: one from a known case of cancer; one from a normal person, and two for diagnosis.

4. Proceed with the reaction as described in the text.

(a) Discuss the principles of this reaction.

(b) Discuss the practical value of this reaction in the diagnosis of cancer.

EXPERIMENT 134.—COLLOIDAL GOLD REACTION:

1. Prepare a reagent as described in the text.

2. Secure four or more specimens of spinal fluid from the following types of cases:

(a) From a case of paresis.

(b) From a case of locomotor ataxia or some other type of tertiary syphilis.

(c) From a non-syphilitic individual.

(d) From a case of suppurative meningitis.

3. Conduct tests with each fluid after the method given in the text.

(a) What constitutes a satisfactory reagent?

(b) Why must 0.4 per cent. sodium chlorid be employed for the dilutions of spinal fluid?

(c) Would the presence of blood in the spinal fluid spoil the reaction?

(d) What types of curves of precipitation of gold may be observed?

(e) Discuss the practical diagnostic value of the test.

ANAPHYLAXIS

For experiments in anaphylaxis sensitize a series of animals as follows:

(a) Give 7 young guinea-pigs an intraperitoneal injection of 0.01 c.c. horse-serum (1 c.c. of a 1 : 100 dilution).

(b) Give 3 young guinea-pigs an intraperitoneal injection of 0.01 c.c. of human serum.

(c) Give 2 young guinea-pigs an intraperitoneal injection of 0.1 c.c. egg-albumen (1 c.c. of 1 : 10 dilution).

(d) Give 2 rabbits 1 c.c. of horse-serum intravenously every three days for three doses.

(e) Give a dog 10 c.c. of horse-serum subcutaneously.

EXPERIMENT 135.—ANAPHYLAXIS IN THE GUINEA-PIG. SPECIFICITY OF ANAPHYLAXIS:

1. Twelve days after the sensitizing injections proceed as follows:

(a) Inject a guinea-pig sensitized to horse-serum with 1 c.c. horse-serum intraperitoneally.

(b) Inject a guinea-pig sensitized to human serum with 0.1 c.c. of human serum intravenously.

(c) Inject a guinea-pig sensitized to egg-albumen with 1 c.c. of diluted (1 : 4) albumin intraperitoneally.

(d) Inject a guinea-pig sensitized to horse-serum with 1 c.c. of diluted (1 : 4) egg-albumen.

- (e) Inject a guinea-pig sensitized to human serum with 0.1 c.c. horse-serum intravenously.
 - (f) Inject a guinea-pig sensitized to horse-serum with 1 c.c. human serum intraperitoneally.
2. Carefully study the symptoms of anaphylaxis. After death autopsy the animals.
- (a) Describe acute anaphylactic shock in the guinea-pig. Does death always occur?
 - (b) How soon after the intraperitoneal injection of the intoxicating dose do symptoms develop? After the intravenous? Why the difference?
 - (c) Is anaphylaxis a specific reaction?
 - (d) What are anaphylactogens?
 - (e) Discuss the prevailing views regarding the mechanism of the anaphylactic reaction.
 - (f) Describe the anatomic changes occurring in acute and fatal anaphylaxis in the guinea-pig. Discuss the cause and nature of these changes.

EXPERIMENT 136.—ANAPHYLAXIS IN THE DOG:

1. Five weeks after sensitization anesthetize a dog with ether and connect the carotid or femoral artery with a mercurial manometer and arrange for a kymographic tracing.
 2. After recording the normal blood-pressure tracing, inject 5 c.c. of homologous serum (horse) into a jugular vein.
 3. Record the blood-pressure.
 4. Study the coagulation time of the blood.
 5. Autopsy.
- (a) Describe the blood-pressure changes. To what may they be ascribed?
 - (b) Is blood coagulation delayed? Give a reason for this change.
 - (c) Do anatomic changes occur in anaphylaxis of the dog?

EXPERIMENT 137.—PASSIVE ANAPHYLAXIS:

1. Secure 1 c.c. of serum from a rabbit sensitized to horse-serum five weeks previously and inject 0.5 c.c. intraperitoneally into each of two guinea-pigs.
 2. Twenty-four and forty-eight hours later inject both animals intravenously with 0.2 c.c. of horse-serum.
- (a) Are anaphylactic symptoms in evidence?
 - (b) Discuss the nature of passive anaphylaxis.
 - (c) If allowed to live, would these animals become sensitized to rabbit serum?

EXPERIMENT 138.—ANTIANAPHYLAXIS:

1. Eight days after sensitizing a guinea-pig with horse-serum, inject 1 c.c. of horse-serum subcutaneously.
 2. Fifteen days after sensitizing a guinea-pig with horse-serum, inject 2 c.c. of serum into the rectum.
 3. Fifteen days after sensitizing a guinea-pig with horse-serum, inject 0.0001 c.c. serum subcutaneously every fifteen minutes for six doses.
 4. On the sixteenth day after sensitizing, inject a guinea-pig with 1 c.c. of horse-serum. If this animal develops anaphylactic shock, inject the other 3 guinea-pigs in a similar manner.
- (a) Do anaphylactic symptoms develop? If not, why not?
 - (b) Discuss the importance of quantitative factors in producing anti-anaphylaxis.
 - (c) Discuss the importance of anaphylaxis and antianaphylaxis in serum therapy.
 - (d) What method is generally employed in the effort to induce an anti-anaphylactic state in serum therapy?

- (e) What drug has been found experimentally of value in ameliorating or preventing the pulmonary changes of anaphylaxis?
- (f) Do anesthetics prevent anaphylaxis?
- (g) Under what conditions would you expect anaphylaxis in human beings?

EXPERIMENT 139.—LOCAL ANAPHYLACTIC REACTIONS; ANAPHYLAXIS IN THE RABBIT:

1. Shave the abdomen of the rabbit sensitized to horse-serum five weeks after the time of sensitization.
2. Make two superficial abrasions over the upper portion of the abdomen; into one rub horse-serum, and in the other normal salt solution.

Does a local reaction occur? If so, describe the lesion.

3. Then inject 0.01 c.c. of serum subcutaneously. If acute anaphylactic death does not follow, observe the animal during the next twenty-four hours.

(a) Does a local Arthus reaction follow this injection of serum? If so, describe the reaction.

(b) Explain the mechanism of a local anaphylactic reaction.

(c) Are the tuberculin, luetin, and mallein reactions anaphylactic? Explain their mechanism and discuss their practical value in diagnosis.

4. Inject 1 c.c. horse-serum intravenously. If anaphylaxis occurs, record the symptoms. Autopsy with particular attention to the heart.

EXPERIMENT 140.—ANAPHYLAXIS IN VITRO:

1. Sensitize a young female guinea-pig with horse-serum.
2. Twelve to sixteen days later remove the uterus and suspend in a saline bath according to the Schultz-Dale method. Arrange for kymographic tracing of contracting uterus.
3. After securing a tracing of normal contractions add horse-serum to the bath and observe the contractions.

(a) Discuss the prevailing views on the humoral and cellular theories of anaphylaxis.

(b) Discuss the nature of anaphylactogens.

EXPERIMENT 141.—CHEMOTHERAPY: TOXICITY OR ORGANOTROPISM:

1. Prepare a 2 per cent. alkalinized solution of arsphenamin by dissolving 0.2 gm. in 8.4 c.c. of hot water and adding 1.6 c.c. of N/1 solution sodium hydroxid.
2. Inject 4 white rats weighing 100 to 150 grams intravenously with the following amounts per 100 grams of weight: 0.4, 0.5, 0.6, and 1 c.c. which administers 80, 100, 120, and 200 mg. of the drug per kilo of body weight.
3. Prepare a 1 : 1000 dilution of mercury bichlorid by dissolving 0.1 gm. in 100 c.c. of saline or distilled water.
4. Inject 4 white rats intravenously with 0.2, 0.4, 0.6, and 1 c.c. which administers 2, 4, 6, and 10 mg. per kilo of body weight.
5. Observe the animals for ten days.

(a) What is the maximum tolerated dose of arsphenamin? Of mercuric bichlorid?

(b) What is meant by toxicity or organotropism?

(c) What is meant by *dosis lethalis* and *dosis tolerata*?

EXPERIMENT 142.—CHEMOTHERAPY: PARASITOTROPISM:

1. Infect 4 white rats by injecting each intraperitoneally with 0.2 c.c. of the citrated blood of rat infected with *T. equiperdum* and showing large numbers of the parasites in the tail blood when examined microscopically.

2. Twenty-four hours later inject No. 1 intravenously with 0.2 c.c. of a 0.5 per cent. alkalinized solution of arsphenamin (freshly prepared by dissolving 0.1 gm. of the powder in

19 c.c. of hot water and adding 1 c.c. of N/1 solution sodium hydroxid) per 100 grams of weight, which corresponds to 10 mg. per kilo of body weight. This amount corresponds to about one-tenth the toxic dose.

3. Inject the second rat with 0.2 c.c. of a 1 per cent. solution of neo-arsphenamin (freshly prepared by dissolving 0.1 gm. in 10 c.c. of sterile water) per 100 grams of weight, which corresponds to 20 mg. per kilo of body weight.

4. Inject the third rat with 1 c.c. of a 1 : 5000 dilution of bichlorid of mercury per 100 grams of weight which corresponds to 2 mg. per kilo of body weight. This amount corresponds to about one-third the toxic dose. The fourth rat is kept as a control.

5. Examine the tail blood of the four rats a few hours later and again at daily intervals.

- (a) What is meant by parasitotropism?
- (b) Is arsphenamin trypanocidal? Is mercury bichlorid?
- (c) What is the purpose of chemotherapy?
- (d) Discuss the question of chemoreceptors.
- (e) Discuss "drug fastness."

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